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# THE OCCURRENCE OF PLEUROPNEUMONIA-LIKE ORGANISMS IN MATERIAL FROM THE POSTPARTUM UTERUS, SIMPLIFIED METHODS FOR ISOLATION AND STAINING

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The occurrence of pleuropneumonia-like organisms in the normal and pathologic vagina and cervix has been verified by the reports of a number of investigators (1, 2, 3, 4). In a recent review on the role of these bacteria in genitourinary and joint diseases, Dienes and his associates (5) conclude that "the relatively high incidence (26%) of this organism in the female genital tract suggests that it is a part of the bacterial flora in this location". The occurrence of pleuropneumonia-like organisms in the postpartum uterus, although hitherto unreported, is therefore not surprising. The primary object of this report is to describe simplified methods for the isolation and staining of these organisms. It is our intention to describe these techniques in some detail, since it has been our experience that although the literature contains excellent morphological descriptions, technical directions for the isolation and staining of these organisms are often so indefinite and incomplete or complicated that the progress of the novice in this field is seriously impeded.

In the course of a study of the effect of penicillin on the bacterial flora of the postpartum uterus (Guilbeau, Schaub and Andrews, 6) cultures were taken from the uterine cavity of 112 postpartum patients, 80 of whom had received varying amounts of penicillin and 32 of whom were untreated. Material from the uterus was obtained by means of a new technique (Guilbeau and Schaub, 7) which we have reason to believe eliminates contamination with cervical and vaginal flora.

The incidence of pleuropneumonia-like organisms in these uterine cultures is shown in Table I. These organisms were isolated from 17 of 112 cases (15.1%), in 11 of which they occurred in pure culture. A marked difference will be noted in the incidence of these organisms in cultures from untreated patients and in cultures from patients who

had received penicillin. This may be explained by the fact that penicillin was demonstrated to eliminate sensitive organisms from all but 18 of the treated cases, resulting in 39 sterile specimens and 11 from which only penicillin-resistant pleuropneumonia-like organisms were isolated. In the control series of 32 untreated patients, only 2 cases were sterile, all others yielding numerous bacteria. We believe the reduction in the number of concurring bacteria in the material from patients receiving penicillin to be responsible for the larger number of pleuropneumonia-like organisms isolated from such cases, since it has been pointed out by Dienes (8) that the "demonstration of pleuropneu-

TABLE I

*Incidence of Pleuropneumonia-like Organisms in Cultures from the Postpartum Uterus*

	ISOLATED FROM UNTREATED PATIENTS	ISOLATED FROM PATIENTS RECEIVING PENICILLIN	TOTALS
Occurred in pure culture	0	11	11
Occurred with other bacteria	1	5	6
Total number of organisms	1	16	17
Total number of cases	32	80	112
Percentage occurrence	3.1%	20.0%	15.1%

monia-like organisms is usually not possible in the presence of abundant bacterial growth"

Dienes (8, 9) has reported the production in vitro of pleuropneumonia-like forms from other bacteria in the presence of penicillin. We have considered the possibility that the pleuropneumonia-like organisms isolated from patients receiving penicillin may represent variant forms of other bacteria, for example *Bacteroides*, which have been produced in vivo by the action of penicillin. However, this theory does not seem to be tenable, since all but two of the strains in our series were carried in pure culture, some for over six months, without reversion to other bacterial forms, and all were identified by stained agar preparations as recommended by Dienes (8) for the accurate morphological identification of these organisms. Culturally and morphologically all strains corresponded exactly with descriptions of pleuro-

pneumonia-like organisms published by Sabin (10) and Dienes (11), which descriptions will not be repeated here

#### METHODS OF ISOLATION

The material from the uterine cavity was inoculated immediately into fluid thioglycollate medium containing 0.5% dextrose and 0.0001% resazurin. To the medium was then added 0.1 cc of a sterile 4% solution of clorase (final concentration 0.25%) to neutralize any penicillin which might be present in the inoculum, and 2 cc of sterile ascitic fluid (final concentration 20%) to enhance the growth of the pleuropneumonia-like organisms. The first three strains were obtained unexpectedly without enrichment of the thioglycollate medium with ascitic fluid. In these instances, after 48-72 hours incubation, the original thioglycollate cultures, which appeared to be sterile, were streaked routinely on blood agar plates and incubated aerobically and anaerobically, and from both sets of plates pleuropneumonia-like organisms were isolated in pure culture. All other strains were isolated by the following method:

The thioglycollate medium containing the material from the uterine cavity, and enriched with 20% ascitic fluid, was incubated for 24 hours. Gram stained smears from such cultures never revealed any forms which could be interpreted as representing pleuropneumonia-like organisms, nor was there gross evidence of their presence, since the original inoculum usually produced marked clouding of the medium and any slight increase in turbidity due to the growth of these organisms could not be detected. In the majority of cases the thioglycollate culture appeared to be sterile microscopically, in others only concurring bacteria, streptococci, Gram negative bacilli, etc., were seen in the smears.

After 24 hours incubation, and daily thereafter, the original thioglycollate cultures were streaked on plates of pancreatic digest agar (Brown, 12) containing 20% ascitic fluid. In preparing these plates, care was taken to use 13-14 cc of ascitic agar to each standard petri dish, so that good agar blocks approximately 2 mm thick could be cut from the plates for staining and transfer, as will be described later. Such plates have been used successfully, for both isolation and maintenance of stock cultures, after being stored in the refrigerator for as

long as two weeks It has been our practice to divide the ascitic agar plates into six pie-shaped segments, using a wax pencil and marking the bottom of the petri dish accordingly A segment was inoculated daily from the original thioglycollate culture from a specific case Thus one week's subcultures were contained on a single plate, which could be examined rapidly for the typical colonies of pleuropneumonia-like organisms The inoculated ascitic agar plates were incubated in a glass jar fitted with a loose glass lid, and in the bottom of the jar were placed several moist paper towels In order to prevent the growth of molds in the jar, the paper towels should be moistened with a non-volatile disinfectant such as a benzalkonium chloride (Roccal or Zephiran) The plates were examined daily under the low power of the microscope for the presence of pleuropneumonia-like colonies, the plate being inverted on the stage of the microscope and the light reduced by lowering the condenser

By means of the daily inoculation of ascitic agar plates, pleuropneumonia-like organisms were demonstrated to be present in the thioglycollate cultures after one to four days incubation Eight strains were isolated after 24 hours incubation, five after 48 hours, three after 72 hours and one strain required 4 days incubation of the thioglycollate medium before growth was obtained Because of this variation in the time required for the original growth of these organisms, daily inoculation of ascitic agar plates is recommended No isolations were accomplished after 96 hours, but subcultures were made routinely for one week before the original thioglycollate culture was regarded as negative for pleuropneumonia-like organisms

On 20% ascitic agar plates, growth was obtained in 24-72 hours, the majority of strains requiring two days incubation Generally the plates appeared to be sterile macroscopically, although in a few instances tiny, pin-point colonies could be detected Microscopic examination, however, usually revealed a heavy growth of the typical colonies of these organisms, although occasionally only a few colonies were obtained The colonies varied in size according to their distribution on the plate, being extremely small in crowded portions, and according to the time of incubation No difficulty was encountered in recognizing the colonies microscopically, although the medium frequently contained considerable precipitate and was milky due to the addition of a large amount of turbid ascitic fluid

In addition to the 20% ascitic agar plates, 10% human blood, pancreatic digest agar plates were also streaked from the 24-48 hour old thioglycollate medium and incubated aerobically and anaerobically. Good growth was obtained on these plates with some strains of pleuropneumonia-like organisms. On aerobic blood agar plates nine strains grew well, five failed to grow and three were overgrown by other bacteria. Anaerobically 10 strains grew on the blood agar, four failed to grow and three were overgrown as on the aerobic plates. It is evident that blood agar plates are less satisfactory for the isolation of these organisms than the 20% ascitic agar plates, on which good growth of all strains was obtained. However, the blood agar plates, particularly those incubated anaerobically, have the advantage of producing larger colonies which can be detected macroscopically. They are very tiny, transparent, pin-point, non-hemolytic colonies. Direct microscopic examination of these colonies is difficult, but they can be studied if the lid of the petri dish is removed and the surface of the blood agar examined directly with the low power objective. The colonies appear in a different focal plane from the red cells in the medium and usually can be recognized as those of pleuropneumonia-like organisms.

#### STAINING OF COLONIES

Dienes (8) has emphasized that only by the examination of stained colonies can pleuropneumonia-like organisms be accurately identified. Due to the delicate nature of these organisms, stained preparations made by the usual technique of fishing colonies are useless, since they show no recognizable organisms. Klieneberger and Smiles (13) have described an agar-fixation technique which is no doubt an excellent research tool, but which is quite impractical for routine diagnostic work. The method of preparing stained agar blocks as described by Dienes (14) was found to be fairly satisfactory but to have certain limitations. In this technique, a small block of agar containing colonies is cut from the plate, placed on a slide, covered with a coverslip on which an alcoholic solution of methylene blue has been dried, and the space between the slide and coverslip filled with paraffin. We found that this method usually resulted in inadequate and uneven staining of colonies, and precipitate from the stain often spoiled the preparations. These difficulties were overcome by using the staining technique employed by Brown and Nunemaker (15), in which Way-

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son's stain is applied directly to the surface of the agar. This stain gives intense staining of the pleuropneumonia-like organisms and results in clear, evenly stained colonies.

The main difficulty encountered in making these preparations, however, was in sealing the coverslip and slide with the melted paraffin. We found it almost impossible to accomplish this without moving the coverslip, and the slightest disturbance of or pressure on the coverslip results in distortion of the soft elements making up the pleuropneumonia-like colony, and colonies may be so damaged as to be almost unrecognizable. Yet in the Dienes method all preparations must be sealed by paraffin in order to support the coverslip during microscopic examination. These difficulties have been eliminated by the use of the following simple technique, which is quick, convenient and gives excellent results with no distortion of the pleuropneumonia-like colonies.

#### STAINING TECHNIQUE

- 1 Make four small balls of plasticine, approximately 2-3 mm in diameter, and place them on a glass slide so as to form the corners of a 1 cm square. The plasticine should be new and soft. We have found that best results are obtained by keeping the plasticine in the incubator or by warming slightly before use. These plasticine balls are to support the coverslip and fasten it to the slide, so that the pressure and slipping which causes distortion of the pleuropneumonia-like colony can be eliminated.

- 2 A block of agar approximately 5 mm square is cut from an ascitic agar plate from an area suspected of containing pleuropneumonia-like organisms. It is important to choose an area containing no colonies of other bacteria, since they are usually washed over the surface of the agar when the stain is applied and obscure the colonies of pleuropneumonia-like organisms which, being imbedded in the agar are not disturbed by the spread of the stain. A thin platinum spatula, 5 mm wide, is used to cut out the block and to transfer it to a glass slide. The agar should be approximately 2 mm thick to facilitate handling without breaking or bending the block, and to allow easy sealing of the preparation with paraffin, as will be described.

- 3 The agar block is carefully lifted from the plate and gently placed

colony side up, on the slide in the center of the square formed by the plasticine balls

4 A small, 1 mm loop of Wayson's stain is then placed in the center of the agar block The formula for this stain is as follows

Dissolve 0.2 gm of basic fuchsin and 0.75 gms of methylene blue in 20 cc of absolute alcohol Add the dye solution to 200 cc of a 5% solution of phenol in distilled water Filter

The intensity of the staining reaction varies with the amount of stain used in proportion to the surface area of the agar block Larger blocks require more stain for adequate staining of colonies

5 Place a clean coverslip over the agar block so that its four corners are supported by the balls of plasticine The coverslip should rest slightly above the surface of the agar Now gently press down on the coverslip, using a blunt instrument and exerting the pressure directly over the pieces of plasticine Press down on opposite corners in rotation In this manner the coverslip is lowered until contact is made with the surface of the agar, which is evidenced by the spread of the stain from the center to the periphery of the agar block If the plasticine is soft and pressure is applied gently and carefully, the coverslip will not be broken, no pressure will be applied to the surface of the agar to cause colony distortion and there will be no slipping of the coverslip to damage the delicate pleuropneumonia-like organisms

The preparation is now ready to be examined microscopically under the oil immersion objective Care must be taken in bringing the colonies into focus, since it has been found that slight pressure of the lens, even on stained preparations, will cause distortion of the elements composing the pleuropneumonia-like colonies

6 After the preparation has been examined and found to be satisfactory, and if it is desired to preserve the specimen, then it may be sealed with melted paraffin We have found it convenient to keep the paraffin in a 20 cc syringe equipped with a 19 gauge needle The syringe and needle can be gently heated to melt the paraffin, which must be quite hot for easy handling Gently insert the heated needle between the coverslip and slide and allow the melted paraffin to fill the space around the agar block If the block is sufficiently thick (about 2 mm) no difficulty will be experienced in manipulating the needle without disturbing the coverslip, which is held firmly in place

by the plasticine. It is usually necessary to fill the four sides of the square separately, since the plasticine prevents the spread of the paraffin completely around the block of agar. When the paraffin has hardened, the excess on the slide can be removed with a knife and a neat preparation results which, if completely sealed, can be preserved indefinitely in its original condition.

This staining method has given excellent results, with good differentiation of the pleomorphic elements composing the mature colony of pleuropneumonia-like organisms, and with no distortion of these elements. For routine identification purposes no troublesome sealing with paraffin is necessary and well stained preparations with intact colonies may be rapidly prepared for microscopic examination. Preparations made from ascitic agar plates are the most satisfactory, but stained blocks may also be prepared from blood agar plates. The presence of red blood cells produces a rather dark field, but the cells themselves do not interfere since they are found in a different focal plane from the pleuropneumonia-like colonies, and the intense staining achieved with Wayson's stain makes the elements composing these colonies visible against the dark background of the blood agar.

#### MAINTENANCE OF STOCK CULTURES

Pure cultures of the pleuropneumonia-like organisms isolated from material from the uterine cavity of postpartum patients were maintained in pancreatic digest broth containing 20% ascitic fluid or on 20% ascitic fluid pancreatic digest agar plates. Broth cultures were inoculated by cutting a small block of agar containing colonies from an ascitic or blood agar plate and transferring the agar block to the ascitic broth. Increase in the turbidity of broth cultures was never noted in positive cultures, nor could recognizable organisms be seen in Wayson-stained smears. In order to demonstrate the presence or absence of growth, it was necessary to streak the broth cultures, after 48-72 hours incubation, on ascitic agar plates on which recognizable colonies could be obtained. Broth cultures were transferred weekly and subcultures checked for growth by streaking ascitic agar plates. A number of strains were preserved for several months in this manner, but the procedure proved too time consuming and was discarded in favor of ascitic agar plates alone.

In transferring pleuropneumonia-like organisms from the original ascitic or blood agar plates to fresh ascitic agar plates, and in the continued maintenance of strains on this medium, the following technique, as described by Dienes (11) and Brown and Nunemaker (15) was used. A block of agar containing colonies was cut from the plate to be subcultured with a platinum spatula and immediately inverted on the surface of a sterile ascitic agar plate. The block of agar was then carefully pushed over the surface of the agar. The area inoculated should be confined to one half the plate, so that the distribution of colonies is restricted for easier microscopic examination. The uninoculated half of the plate may be used for the next subculture. Stock cultures of a number of strains of pleuropneumonia-like organisms have been maintained in this manner for six months. The only difficulty to be encountered was in contamination of the plates with fungi, which occurs all too frequently during incubation in the moist jars. To prevent loss of cultures through such contaminations, stock cultures should be maintained in duplicate and transferred every three to four days.

#### SUMMARY

Using a simple procedure based on the routine method for the isolation of bacteria in general, seventeen strains of pleuropneumonia-like organisms have been isolated from material from the postpartum uterus. The material was inoculated into thioglycollate medium containing 20% ascitic fluid and streaked after 24-48 hours incubation on aerobic and anaerobic blood agar plates. In addition 20% ascitic agar plates were inoculated daily and from these ascitic agar plates the largest number of pleuropneumonia-like organisms were isolated.

A simplified technique for staining colonies of these organisms has been described.

The significance of pleuropneumonia-like organisms in the postpartum uterus is not known at the present time. They appear to be part of the normal flora of the vagina and cervix, but Dienes et al (5) have presented evidence that occasionally they may have some pathogenic action in the female genital tract. In our series, the patients from whom these organisms were isolated had an uneventful clinical course, which suggests that in these cases the pleuropneumonia like organisms may be regarded as non-pathogenic.

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# SUBUNGUAL HEMORRHAGES AND HYPERKERATOSIS DUE TO EVERON

MAURICE SULLIVAN

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Fingernail polish is well known as a cause of allergic contact dermatitis (1) In persons hypersensitive to one or more of the ingredients of nail polish, plaques of erythematous, edematous, scaly dermatitis may be observed on the eyelids, chin, sides of the neck, cheeks, thighs or any other areas of the skin readily reached by and frequently in contact with the fingernails Fingernail polish dermatitis never occurs on the fingers nor is there evidence of acute inflammation of the nails, it is, therefore, an example of a finger borne contact dermatitis with a diagnostic distribution in telltale areas remote from the application site No one ingredient has been implicated as the cause of every case of nail polish dermatitis The plasticizers, fixatives, dyes and perfumes separately or in combination have been incriminated

At the Round Table Discussion of the 1948 meeting of the American Dermatological Association, several members from different sections of the United States reported a new entity affecting the fingernails, it is caused by the application of nail bases These are cosmetic preparations which are applied to the surface of the nail prior to the application of the lacquer to insure the adherence of the lacquer Cases were reported from New York City by Sulzberger, Sharlit and Rein; from Chicago by Mitchell, and from Los Angeles by Ayares The widespread use of nail bases makes it imperative to disseminate rapidly information regarding this new entity, as it can be confused with onychomycosis, psoriasis, verrucae and other subungual tumors, results of trauma to the nail bed, and a manifestation of blood dyscrasia If the condition remains unrecognized the patient is likely to continue the use of the nail base in an effort to cover up the unsightly appearance of the nails and thus further aggravate the condition In severe cases there is often excruciating pain and surgical removal of the nails is necessary in some instances The following case due to a nail base, Everon, is reported because it is illustrative and corresponds to the description of other cases reported at the A D A Round Table

## CASE REPORT

Mrs R E White aged 42, consulted me on 18 May 1948 with the complaint of discoloration and deformity of the fingernails and pain in the finger tips. There was no history of skin diseases in the past. Her general health was excellent. For many years she had used and tolerated Revlon nail polish. On the morning of 5 March 1948 a beauty parlor operator applied Everon for the first time. This base



FIG 1

was then covered in the usual manner with Revlon nail polish. That night, according to the patient, she experienced a painful sensation under one fingernail. She attached no significance to the pain and one week later she returned to the beauty parlor, where another application of Everon was made. Again Revlon was applied on top of the nail base. That night and for several days thereafter she experienced increasing pain under all of the nails and when she finally removed the nail polish she noted a purple-brown discoloration under all of the nails. Above the

discolored area, beneath the nail, a lighter brown material had accumulated. This material grew upward and pushed itself above the edge of the nail. She consulted several physicians who were not acquainted with the condition and it was their consensus that she had onychomycosis. Many scrapings of the material beneath the nails and cultures on Sabouraud's medium failed to support the diagnosis, and treatment for fungus infection of the nails was ineffective. She consulted me nine weeks after the onset.

*Examination* The fingernails were universally involved by a process (See Figure 1) consisting of onycholysis of the upper half of each nail, thick hyperkeratoses (See Figure 2) of the nail bed under the area of onycholysis and wide



FIG 2

bands of brown discoloration across the middle and lower portion of each nail. Punctate hemorrhages were present on the borders of the bands. There was slight edema of the terminal phalanx of each digit.

Patch tests with Revlon and Everon were performed on 18 May 1948. There was no reaction to Revlon, but there was a markedly positive 48 hour reaction to Everon, consisting of a plaque of erythematous, edematous, vesicular inflammation which persisted until 10 June 1948.

On 17 June 1948 patch tests were performed with three other brands of nail bases: namely, Foolproof, Strizon and Perma-Nail, and with three ingredients of Everon: namely, methyl ethyl ketone, a rubber solution and a resin solution. Markedly positive 48 hour reactions were produced by the three other brands and by the rubber solution.

*Treatment* The patient was apprized of the nature of the disease so that she would avoid further application of Everon or other nail bases. Compresses of



saturated solution of boric acid applied with one inch roller bandages under rubber finger cots were advised. After the compresses had been in place for one hour and there was softening and maceration of the hyperkeratotic material it was possible to cut away a small amount of the protruding hyperkeratosis and to trim the nails. With this simple treatment there was symptomatic relief and in five weeks there was considerable improvement. The brown bands had grown upward and the hyperkeratotic material had been appreciably reduced. The texture of the nails, although longitudinally ridged, was approximately normal.

#### DISCUSSION

When this disease was called to the attention of the American Dermatological Association many of the members commented that they had observed patients with a similar condition but had failed to recognize it. It is likely that many reports of subungual hemorrhages and hyperkeratoses will soon appear. It is probable that the nail bases, when applied, are not precisely confined to the outside of the nail and that small quantities of the liquid spill behind the nail and gain access to the under surface. This condition differs considerably from nail polish dermatitis, in that inflammation at remote finger born sites is not observed. It is likely that the protection of the overlying nail lacquer prevents the nail base from causing inflammation to the eyelids, chin, neck and the sites characteristically implicated by nail polish. In an individual hypersensitive to nail polish, however, it would be possible to have a combination effect. Mitchell of Chicago is preparing an exhibit on the subject for the forthcoming American Medical Association Meeting. Through this medium many physicians will become acquainted with the entity and will save unnecessary discomfort and suffering.

#### SUMMARY

The case of a patient with subungual hyperkeratoses and hemorrhages due to Everon is presented.

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# RECOLLECTIONS OF WILLIAM STEWART HALSTED\*

## A GREAT SURGEON

S J CROWE

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My father was born on a farm in Washington County, Virginia. He received a college education and went to the College of Physicians and Surgeons in New York, was an interne in Bellevue and attended Dr Welch's lectures. He never knew either Dr Welch or Dr Halsted intimately, but was much impressed with the new medicine these two men had brought from Europe. On account of my mother's health he moved to Atlanta. The South was impoverished, and he had to work very hard to make a living, doing everything—general practice, obstetrics, and some surgery. As I grew up my impression of medicine was that it was a most undesirable profession. My father was always tired, pale and thin. He was often called out at night on obstetrical cases. I never knew him to take a vacation—and the summers are very long and hot in Georgia. Medicine was the one profession I never wanted to enter.

At the University of Georgia, the Professor of Physics interested me in the future of electricity and I was determined to become an electrical engineer. My father's one idea was that I should study medicine, return to Atlanta and gradually take over his practice. I never discussed my ambitions with him, but all my activities at the University were devoted to athletics and courses that would enable me to enter the Georgia Tech. No more biology or premedical work was taken than was absolutely necessary for me to get an A B degree. My father knew nothing of this. It never entered his head that I would not become a doctor. After graduation I realized for the first time how much he wanted me to study medicine. In that conversation I heard of the Johns Hopkins Medical School for the first time. His chief desire in life was that I should go to Baltimore and come under the influence of Dr Welch, Dr Osler and Dr Halsted. I could not refuse.

How I got into the Hopkins Medical School I will never know. I

\*Read at a meeting of the Surgical Staff of the Johns Hopkins Hospital in May 1948

came to Baltimore in 1904 much against my will and with no interest in medicine. In some way I managed to pass at the end of the first year. During the summer following my graduation at the University of Georgia, I had joined two friends on a camping trip in the North Carolina mountains. We hired a covered wagon and a team of horses and drove aimlessly through the mountains toward Asheville—with no maps or guides. We slept in the wagon and tied the horses to trees. It rains in those mountains almost every day, but there were no mishaps and the six weeks trip was a great success. As soon as my first year in the medical school came to an end I gave a great sigh of relief and hurried to Georgia to organize another camping trip in the North Carolina mountains. This was an unusually cold and rainy summer, but we reached Highlands, North Carolina, stayed in that lovely country a few days, letting the horses graze, then moved on in the general direction of Asheville. After an unusually cold rainy night I got up the next morning to feed the horses and found that one of them—a big Percheron—was standing with his front legs far apart and his head hanging. He was as stiff in every joint as if he had been made of wood. I was amazed. On my grandfather's farm in Virginia I had seen horses turned out to graze in any kind of weather. I thought they were as immune to weather as a tree, so of course had brought along no horse blankets. I was also much disturbed to be in this wild country, so far from home, with a hired wagon and two valuable horses and one of them sick. Without waiting for breakfast, a shave or a wash up, I got on the well horse and leading the other at a snail's pace went down the road in search of a cabin, a barn, and some advice about what to do for a stiff horse. I met no one, but finally came to a side road and saw a cabin in the distance. An old mountaineer was sitting on the porch, so I told him my trouble and asked where I could find a barn for my horse. His horse was about the size of a donkey and his barn about the size of a large chicken coop. He said all other barns in that part of the country were about the same size. I was truly in despair, but after a lot of useless talk he finally said "Doc" Halsted from Baltimore lived about two miles up the road, that he had horses and a big barn with real box stalls.

I mounted the good horse, leaving the stiff one standing in the road and soon came in sight of the big barn, a beautiful, well kept lawn, and

about half an acre of the most gorgeous dahlias I had ever seen. There were two small houses. I knocked at the door of one, and finally a maid in dainty white cap and uniform appeared. She said Dr Halsted was out on the place somewhere. I sat on the porch for about an hour. Then Dr Halsted came out of the house in spotless flannel trousers, patent leather pumps and a white silk shirt. I was much embarrassed because I knew I looked like a tramp and I certainly felt like one. I told him I had just finished the first year at the Johns Hopkins Medical School, was on a camping trip, and about the stiff horse. Instead of saying "go get your horse and put him in my barn", he called a man, had his buggy sent around, and insisted that we drive down and look at the sick horse. That impressed me as being very kind, but I was amazed at what happened after we got to the horse. Dr Halsted got out, walked through the mud in his fine clothes, walked all around the horse, feeling every muscle and joint. Then standing in the middle of the road he asked me to lead the horse. We made slow progress for about fifty feet and returned. This was repeated several times. Dr Halsted was intensely interested and finally said he did not think it was hip-joint disease, but probably muscular rheumatism. At his suggestion I tied the horse to the back of the buggy and we literally crept up the road. On the way Dr Halsted said he thought the horse should be bandaged with oiled silk after a rub down with Sloan's liniment. He had the horse put in a box stall with a lot of straw, gave me some lunch, and I rode off to the country store where I bought all the liniment and oilcloth in the store. The oilcloth such as is used to cover kitchen tables was of all colors, since it took all they had in the store to bandage such a big horse. I bandaged him from his ears to his hoofs. He recovered completely.

I was there a week, but was much embarrassed because I had no fine clothes. I talked very little, but Dr Halsted seemed to like me, and finally I told him my ideas of medicine as a career. He said very little then, but during the days that followed he talked more and more about medicine being a living, growing, constantly changing and most fascinating study. That of course the doctor must know the structure and the physiology of the various organs and how to treat the sick, but that also it was the duty and function of the doctor to seek by clinical and laboratory research to lessen in the next generation the number of

cripples from disease that constantly flow into our hospitals That there was a great satisfaction in restoring a sick person to health, but that there was greater satisfaction if a man was fortunate enough to devise some means to prevent disease That monetary rewards were necessary or advisable only to the extent of allowing the doctor to care for his family, to travel in order to acquire new ideas, and to have the leisure and peace of mind to think and read and try to add something during his lifetime of lasting value to medicine This was in 1905 No doubt it was for these reasons that he enthusiastically welcomed the Full Time Plan when it came along in 1913

That chance meeting in the mountains with Dr Halsted changed my whole life Without it I am sure I would have withdrawn or have been asked to withdraw from the medical school during the second year, because I was not interested, and I was not interested because I didn't understand what opportunities medicine offered for a happy as well as a useful life I have gone into some detail with this story, because all of you may have an opportunity to help some young man in the same way

You can learn from Dr MacCallum's biography of Dr Halsted that the Halsted family in New York was prominent financially and active in various philanthropic projects Dr Halsted entered Yale at the age of eighteen His scholastic record was poor There is no record in the Yale Library that he ever borrowed any books He spent most of his time in athletics—rowing, baseball, gymnastics, and in his senior year was captain of the Yale football team and made the winning goal in the game with Eaton, which Yale won two to one He entered the College of Physicians and Surgeons in New York and became intensely interested in anatomy He graduated with honors in 1877, and received an appointment at both Bellevue and the New York Hospital He spent the next two years in Europe attending courses in anatomy and the clinics of Billroth, Mikulicz, Von Bergmann, Thiersch, Von Volkmann and others, watching the development of Lister's antiseptic surgery in Germany On his return to New York in 1880, he worked with Dr Welch in pathology at Bellevue, inaugurated at the Roosevelt Hospital the first surgical out-patient department in New York, was demonstrator of anatomy in the College of Physicians and Surgeons, visiting physician to Bellevue, The Presbyterian Hospital and the Charity Hospital on Blackwell's island, and Surgeon-in-Chief to the

Emigrant Hospital on Ward's island In addition, he carried on a quiz course at his house on 25th street, and for several years had sixty-five or more students taking the course These many and varied activities attest to his enthusiasm and splendid physical health

It was at this time he became interested in Koller's discovery that the cornea could be anesthetized with cocaine He experimented on himself and found that by injecting this drug into a sensory nerve he could render the whole area from which its branches came insensitive to pain Nearly forty years later at a great banquet at the Belvedere Hotel in Baltimore he was awarded a gold medal by the National Dental Association in recognition of his discovery of local anesthesia and his great service to dentistry

When Dr Halsted was making these experiments on himself in 1885, it was not known that cocaine was a demoralizing, habit-forming drug Through the aid and encouragement of Dr Welch he finally overcame it, but this accident radically changed his whole life He returned to New York after a cure in a Providence Hospital to a more thoughtful and leisurely life, with time for reflection and study of the surgical problems that interested him most, a life far more fruitful than it could ever have been if he had continued the strenuous pace he had set for himself in the beginning

The Johns Hopkins Hospital was opened in May 1889 Dr Welch had accepted the appointment as Professor of Pathology in 1884 In 1886 he invited Dr Halsted to come to Baltimore He lived with Dr Welch at 506 Cathedral Street and in the Pathological laboratory began experimental studies that continued without interruption as long as he lived It was then that he and Dr Mall did their fundamental work on intestinal suture, and during the following year Dr Halsted began his experimental studies of the thyroid in dogs At this time Dr Halsted also began to work experimentally on animals on the basic questions of operative procedures and the healing of wounds He showed that cultures after the most meticulous use of Lister's methods still showed the presence of bacteria He made histologic studies of healing wounds and of tissue reaction to various types of suture material These laborious and careful studies led him to the realization of the great importance of the careful handling of tissues, the necessity of controlling bleeding with the minimum of crushing of tissues with forceps, and

the value of transfixing vessels and ligating them with the smallest and finest silk Also the value of exact approximation of tissues and avoidance of dead space in the healing of wounds

These years of laboratory work were largely responsible for Dr Halsted's appointment in October 1889 as Associate Professor of Surgery and Acting Surgeon to the Hospital He was not made Professor of Surgery until April 1892 He was then 40 years of age His work during the six years he had been in Baltimore and his University appointment show how completely he had overcome the cocaine habit Also his work during these six years, and indeed throughout the remainder of his life, illustrates one of Dr Halsted's most prominent characteristics i e, his ability to select a basic problem and to stick to that problem until it was solved to his satisfaction Dr Dandy had the same gift It was one of the outstanding characteristics of these two men that won for them respect and fame, and one that all young men should emulate

In Baltimore, Dr Halsted never gave a systematic course of lectures His clinics and ward rounds were meant to present some fundamental underlying principle of disease and the surgical treatment of disease in a way that would stir the spirit of an investigator He taught by example more than by text

It was my privilege to see much of Doctor and Mrs Halsted in their home at 1201 Eutaw Place His house was filled with the most beautiful antique furniture and rugs A wood fire always burned in his study He rarely read anything but medical articles and books dealing with some subject on which he was working When writing his paper on "The Operative Story of Goitre" in 1919, he read hundreds of papers bearing on the subject No reference was ever quoted until he has seen the original article His table was covered with dictionaries and grammars in every language and boxes of books from the Surgeon General's library were constantly arriving

When he and Mrs Halsted had guests to dinner, it was the Professor who selected the menu, the china, the wine, and even the table linen I was much impressed late one afternoon to find him in the dining room supervising the ironing of the table cloth after it had been spread on the table All folds and creases had to be removed On another day I found him bandaging with the greatest care the leg of a large plush

tiger that he had bought for the five year old son of one of his friends On the day before the child had fallen and badly skinned his knee

About 1919, he began to have gall stone colic for which a few weeks later he was operated on by Dr Follis For several days he had complained to Dr Welch of pain, but did not localize it or suggest a cause Then one evening when he and Dr Welch were walking up the steps of the Maryland Club, Dr Halsted stopped and, looking very serious and distressed, said "Welch I have found the cause for my pain It's an aneurism Put your hand under my vest and see if you don't think I am right " Dr Welch was amazed to feel the violent, rhythmical pulsations, and became much excited Dr Halsted had spent many hours in having a thin rubber bag made, which he had fastened to his chest wall with adhesive From the bag a small rubber tube ran to his pants pocket where it ended in a bulb While Dr Welch was still palpating the aneurism Dr Halsted gave a great squeeze to the bulb and the bag exploded with a bang

I well remember a few days before Christmas, Dr Halsted came home with about ten pairs of green and yellow carpet slippers that he had bought on Gay Street He wrapped each pair of slippers in many layers of tissue paper, placed them in a Christmassy-looking box and directed the boxes to Dr Thayer, Dr Finney, Dr Bloodgood, Dr Young, Dr Welch and others On each box was pasted a cancelled English, French, German, Austrian or Italian stamp that Dr Halsted had saved for this purpose, and in each box was placed the visiting card of some foreign doctor who had visited the Hopkins Hospital On each card was written in English, French, German or Italian "The Season's Greetings "

I have told you how my sense of loyalty and my love and admiration for my father forced me to go to the medical school in 1904 against my wishes The same sense of loyalty, love and admiration for Dr Halsted diverted me eight years later from my chosen specialty, neurological surgery, into a branch of surgery about which I knew absolutely nothing and at that time regarded as the lowest form of all surgery—otolaryngology

From the time he became Professor of Surgery, Dr Halsted limited his operative work to the maladies that interested him most fracture of the neck of the femur, intestinal anastomosis, hernia, cancer of the



breast, thyroid and parathyroid diseases, surgery of the gall ducts and vascular surgery This was a deliberate plan in order to give his house officers better training, and to give himself more leisure to think, to read and to carry on investigations The period of training for house officers was long—from 12 to 14 years As their knowledge of disease in all its manifestations increased, their responsibilities became greater The Resident Surgeon during the early years of the hospital could do and did do approximately 90 per cent of all the operations As suitable men appeared, Dr Halsted encouraged them to interest themselves in some special branch of surgery Thus, in a gradual and unhurried way, the departments of neurological surgery, genito-urinary surgery, orthopedics, and otolaryngology were built up Of the men selected to head these various departments, only Dr Cushing was kept in line and advanced until he became Resident in Surgery The others were diverted into special branches after they had been in training long enough to master some of the fundamentals of general surgery

All my training had been in the Hunterian Laboratory and in the hospital as Resident in Neurological Surgery Dr Cushing had invited me to go to Boston with him, when without any warning Dr Halsted one day asked me to stay on and try to build up a department of otolaryngology The fact that I had never seen a tonsillectomy, a mastoidectomy or the inside of the nose did not disturb him He simply said the regional anatomy, physiology, pathology and the technical procedures of treatment could soon be learned In the next two years I went abroad frequently for a month at a time for intensive study of some phase of this specialty, but always at my own expense Then came the first World War, and I was on my own This may have been the reason, but at any rate from 1914 to 1924 my enthusiasm for my specialty did not grow and my knowledge of otolaryngology was far from satisfactory to me, and I suspect to Dr Halsted also I spent all of my spare time during those years working in the Hunterian Laboratory on the pituitary gland, on the function of the adrenals with Dr Wislocki, who is now Professor of Anatomy at Harvard, in removing the thymus gland from innumerable newborn kittens for Dr Park, and on lung abscess with Dr Scarff Dr Halsted, however, never criticized or made any suggestions He was always interested, cordial and encouraging He would occasionally come into

the operating room and look over my shoulder when I was doing a tonsil or mastoid operation. He never lived to see the awakening of my interest in otolaryngology that came with the advent of the audiometer, the establishment of the Otological Research Laboratory and a sane method for learning something about the ear and how it functions.

In concluding this talk I wish to say, in agreement with Dr MacCallum, that Dr Halsted's greatest accomplishment lay in the establishment of a school of surgeons, who in turn have passed on to their assistants his spirit of inquiry and desire to learn the cause of disease, his teachings regarding the delicate handling of tissues, hemostasis and asepsis, and his unfailing courtesy and interest in the patient as a human being.

# PENICILLIN IN THE TREATMENT OF EARLY SYPHILIS COMPLICATING HEMOPHILIA\*

## REPORT OF A CASE TREATED WITH 48 MILLION UNITS OF ORAL PENICILLIN G

MINERVA S BUERK AND HAROLD A TUCKER

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An unusual therapeutic problem recently arose in the Syphilis Clinic of The Johns Hopkins Hospital when a patient with hemophilia was found to have untreated early syphilis. Because of his grave hemorrhagic disease, it was believed that parenteral arsenobismuth or penicillin therapy was inadvisable. Although oral penicillin has been considered unsuitable for general syphilotherapy (1), in our opinion the oral route of penicillin administration in the case here reported provided the least objectionable method of treatment at present available.

### CASE REPORT

The patient (J H H 291036) was a 22 year old Negro with known hemophilia, first seen in the Syphilis Clinic of The Johns Hopkins Hospital in March, 1948 as a sexual contact of a patient with gonorrhea. No clinical or bacteriologic evidence of gonococcal infection was found, but repeated serologic tests for syphilis were positive with titers as high as 64 Eagle units. He stated he had had transitory sore throat and "blisters" on his penis one month before. Although a lumbar puncture would have permitted a more explicit diagnosis (i.e., early latent or early asymptomatic neurosyphilis), the procedure carried with it certain risks in this particular case and the examination was foregone. Because of the hemophilia it was decided that oral penicillin therapy was the treatment of choice and the patient was admitted to the ward for syphilotherapy and study.

The maternal grandfather, a white man, and four of the patient's brothers also had hemophilia. Three of the six male siblings had died from hemorrhage at 18, 22 and 37 years of age, the other brother with hemophilia died of poisoning when he was 24 years old. There was no other known syphilitic disease in the family.

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\* From the United States Public Health Service and the Johns Hopkins University Venereal Disease Research and Post-Graduate Training Center, Baltimore 5, Md. The laboratory studies were carried out in the Laboratory of Experimental Therapeutics, School of Hygiene and Public Health, Dr. Harry Eagle, Director.

Since early childhood, the patient had been hospitalized elsewhere on 19 occasions for hemorrhagic episodes of variable severity. In April 1943 and March 1947, he had been admitted to the wards of the Johns Hopkins Hospital because of massive hematomas and hemarthroses. On these admissions he was studied thoroughly from the hematologic standpoint and serologic tests for syphilis were negative.

On physical examination, at the time of the March 1948 admission, the patient was found to be well nourished and developed, he weighed 71.7 kg. The skin and mucous membranes were not remarkable. The posterior cervical, axillary and epitrochlear lymph nodes were enlarged bilaterally, but these, as well as the enlarged right inguinal nodes, were not fluctuant or tender. The blood pressure was 120 mm of mercury systolic, 76 mm diastolic. The heart was normal to percussion and auscultation. The liver extended five cm below the right costal margin on deep inspiration and was firm and nontender. No active lesions or scars were seen on the genitals. There was enlargement of the weight-bearing joints and of the left elbow, with varying degrees of functional impairment. A massive, partially calcified hematoma could be palpated in the anterior aspect of the left thigh. Neurologic findings were normal.

Numerous laboratory studies were carried out. Blood counts, hematologic indices and platelet counts were normal. The non-protein nitrogen, fasting blood glucose, cholesterol, total serum proteins and albumin-globulin ratio, serum calcium and alkaline phosphatase activity were all within the range of normal. The bleeding time was three minutes. The prothrombin time was 19 seconds, which was normal for the method used. The venous clotting time at 37°C was 15 minutes in the first tube, 20 and 33 minutes in the second and third tubes respectively. The Rumpel-Leede test was negative. Five serologic tests for syphilis varied in titer from 24 to 64 Eagle units. Radiographic examination of all the large joints revealed changes characteristic of old hemorrhagic arthropathies.

Throughout his stay in the hospital the patient was given a regular house diet and fluids as desired. The oral penicillin preparation employed was in the form of compressed tablets, each containing 100,000 Oxford units of crystalline sodium penicillin G, with 0.5 gm of trisodium citrate incorporated as an antacid buffer.<sup>1</sup> He received a total dosage of 48 million units, 500,000 units (five tablets) every two hours for a period of eight days. Meals were given midway between doses of penicillin.

No untoward reactions occurred during or after the course of treatment. Rectal temperatures taken every two hours through the first 24 hours of therapy did not show evidence of a febrile Jarisch-Herxheimer reaction. During the period of treatment, serial examinations showed no significant alterations in the hematologic findings. (2) Seventy-four days after the start of penicillin therapy the patient

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<sup>1</sup> The oral penicillin G used in this study was furnished through the courtesy of E. R. Squibb and Sons.

was clinically well from the standpoint of his syphilis and no new clinical manifestations of hemophilia had appeared. The serologic titer was 16 Eagle units.

Special penicillin studies were carried out on this patient. Blood specimens were taken by venepuncture one half, one, two, three, four, eight, 12 and 24 hours after the first dose of oral penicillin. Thereafter, throughout the course of therapy, daily specimens were drawn one hour after breakfast and immediately prior to the next dose of penicillin, with the exception of the ones taken at 168 and 192 hours, breakfast was omitted before these venepunctures. Pooled urine specimens

TABLE I  
*Penicillin Serum Concentrations and Total Urinary Recovery During  
Administration of Oral Penicillin G*

	TIME IN HOURS							
	½	1	2	3	4	8	12	24
Serum*								
Oxford Units	0.42	5.03	0.64	2.19	0.32	0.32	0.42	1.71
Micrograms	0.25	3.01	0.38	1.31	0.19	0.19	0.25	1.02
Urine†								
Oxford Units	4,510	51,600	80,200	53,500	47,400	72,400	112,000	405,000
Micrograms	2,700	30,900	48,000	32,000	28,350	43,300	67,000	242,000
	48	72	96	120	144	168‡	192‡	
Serum*								
Oxford Units	1.06	0.30	0.95	1.29	1.20	4.01	6.02	
Micrograms	0.63	0.18	0.57	0.77	0.72	2.40	3.60	
Urine†								
Oxford Units	441,000	638,000						
Micrograms	264,000	382,000						

\* Penicillin concentration in units and micrograms of penicillin G per cc. of serum

† Penicillin concentration in total pooled urinary output for period ending at time stated (total volume in cc. X concentration per cc.)

‡ Patient chewed the 30 penicillin tablets making up the 6 doses prior to fasting venepuncture

for the corresponding intervals of time were collected during the first three days of treatment. These serum and urine specimens were assayed by one of us (H.A.T.) for penicillin G content using the Eagle modification of the Kirby-Rantz method<sup>3</sup>. The endpoint in this serial dilution technic of penicillin assay was the complete inhibition of hemolysis of human group O erythrocytes by the C-203 strain of *Streptococcus pyogenes* following incubation for six hours at 37°C and then ten to 12 hours more at room temperature.

## RESULTS

In Table I are shown the penicillin G concentrations per cc. of serum at the various time periods after the start of treatment. The mean concentrations of penicillin in the pooled urine specimens multiplied

by the total volume of urine excreted in a given interval are also tabulated for the first 72 hours. These values are expressed both in Oxford units and in micrograms of penicillin G.

All serum assays showed concentrations of penicillin G in excess of 0.30 Oxford units per cc. The post-prandial morning specimens (after the first day) ranged from 0.302 to 1.71 units per cc. of serum and were equivalent to levels obtained after the intramuscular administration of approximately 100,000 to 330,000 units of penicillin G in aqueous solution at intervals of two hours in the average adult (4). These data confirmed numerous reports in the literature to the effect that effective plasma levels may be maintained by the use of oral penicillin, but that approximately five times as much penicillin must be given orally as is necessary parenterally in order to maintain a given plasma concentration (5-9). The total penicillin G recovered in the three 24-hour pooled urine specimens was approximately eight per cent of the daily dosage (6,000,000 units), this likewise resembled results reported by others (6, 8).

#### SUMMARY

Buffered crystalline sodium penicillin G was used for the treatment of early syphilis in a patient with hemophilia. A total of 48 million Oxford units was given in eight days (500,000 units every two hours), and serum and urine assays were carried out during treatment. The plasma concentrations corresponded to those in patients given 100,000 or more units in aqueous solution every two hours by the intramuscular route. By present standards, this probably represents adequate therapy for early acquired syphilis.

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# STUDIES ON A PROTEOLYTIC ENZYME IN HUMAN PLASMA

## IV THE RATE OF LYSIS OF PLASMA CLOTS IN NORMAL AND DISEASED INDIVIDUALS, WITH PARTICULAR REFERENCE TO HEPATIC DISEASE<sup>1</sup>

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In 1914, Goodpasture (1) observed that the clotted blood of four patients with atrophic cirrhosis of the liver dissolved within 16 hours when incubated at 37°C. On the contrary, the blood clots of normal persons did not lyse for many days. Since then, this phenomenon of fibrinolysis has been described under a wide variety of conditions. In an attempt to evaluate its clinical significance, the fibrinolysis of plasma clots has been measured in normal persons and in patients with a wide variety of diseases. Data will be presented which indicate that, under certain specified conditions, rapid fibrinolysis is particularly frequent in patients with hepatic disease, although it may occasionally be present under other conditions as well.

### METHOD

Venous blood was drawn under sterile conditions from the antecubital vein of human subjects and transferred to a 50 cc centrifuge tube containing 0.2 cc of oxalate mixture for each 5 cc of blood added. The oxalate mixture was prepared by dissolving 3 grams of ammonium oxalate and 2 grams of potassium oxalate in 250 cc of water (2). The blood was centrifuged immediately for 20 minutes at 2000 r p m in an angle centrifuge (rim diameter 10 inches) and the plasma separated. Half a cc of plasma was placed in a sterile Wassermann tube 13 mm in diameter and the plasma recalcified with 0.1 cc of sterile M/20 calcium chloride solution. The tube was then closed with a sterile rubber stopper, incubated at 37°C, and observed several times daily for clot lysis. Grossly, the process of clot lysis appeared to begin when the clot lost its firm appearance and seemed friable. In the course of

<sup>1</sup> These studies were conducted under contract with the Office of Naval Research, U S Navy



several hours the clot appeared to melt, usually from the top down, until only a cloudy suspension remained. The contents of the tube

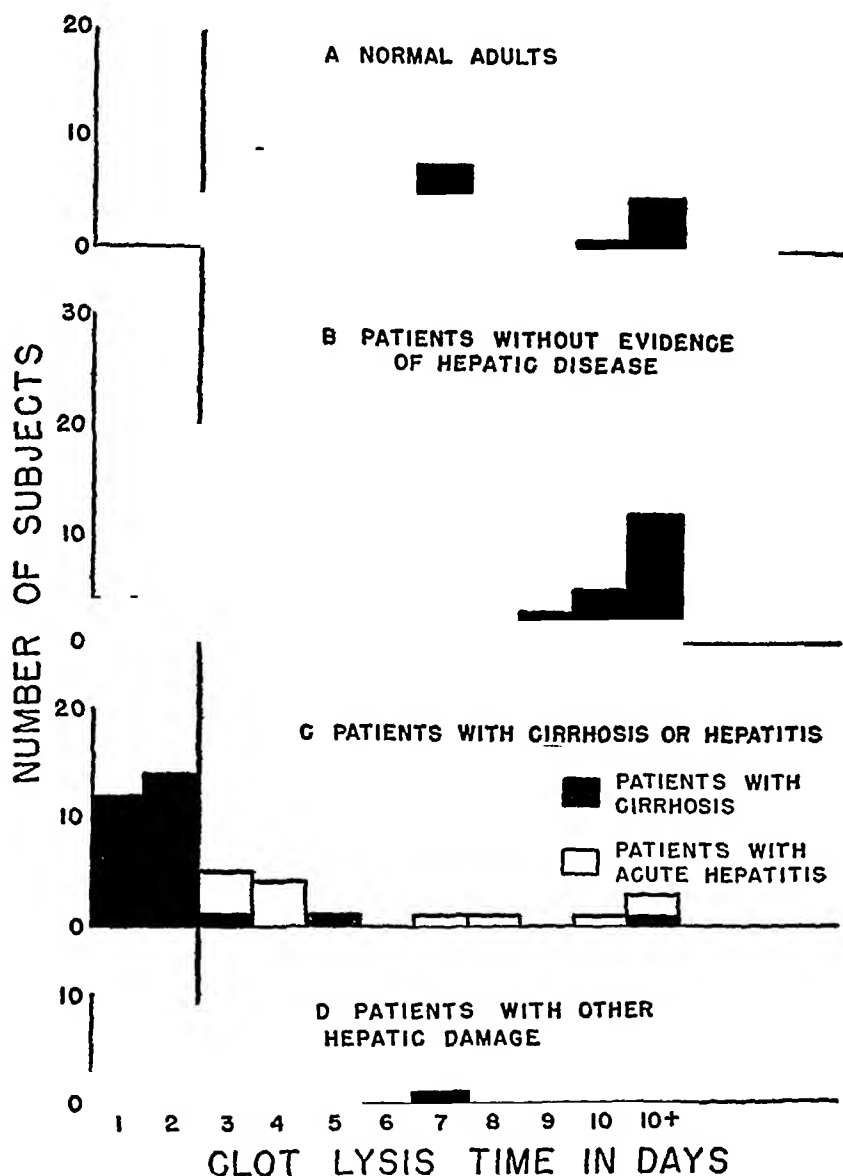


FIG 1 LYSIS TIME OF RECALCIFIED PLASMA CLOTS IN NORMAL AND DISEASED INDIVIDUALS

gradually became clear and a small amount of white sediment could be seen in the bottom of the tube. The length of time from the formation of the clot until the contents of the tube became clear was recorded as

the clot lysis time When lysis occurred in 48 hours or less, the sterility of the tube was verified by culturing its contents on a blood agar plate or on Brewer's anaerobic medium If bacterial contamination was present, the test was not considered valid

Since clot lysis frequently did not occur for many days, it was convenient to record the clot lysis time in days A patient whose clot lysed in less than 24 hours was said to have a clot lysis time of one day, if the clot lysed between 24 and 48 hours, he was said to have a clot lysis time of 2 days, and so forth Frequently, the clots were discarded after 10 days, and the clot lysis time then recorded as 10+ days

#### EXPERIMENTAL

(1) *The Clot Lysis Time in Normal Human Beings* The clot lysis times of 49 presumably normal human adults were determined in the manner described The subjects included 33 men and 16 women, personnel or medical students at the Johns Hopkins Hospital, all of whom were apparently in good health The blood was not drawn in any particular relation to meals or activity

The clot lysis times of the normal adults have been recorded in Fig 1A In these normal human beings, the recalcified plasma clot, when incubated at 37°C, did not lyse in less than three days, and usually not for at least four days The shape of the distribution curve for the clot lysis time of normal adults, however, suggests that occasionally a normal person's clot would lyse within one or two days That this has not yet been observed may indicate that the sample is too small However, under the conditions of this study, fibrinolysis was not observed in normal human adults in less than three days

The clot lysis time of a number of subjects was determined repeatedly In a small group of normal men, the clot lysis time did not usually vary more than a day or two from time to time In women, on the other hand, considerable variation was noted from week to week Thus, in one normal woman, the clot lysis time varied from 8 to 40 days, in another from 5 to 28+ days, and in a third from 4 to 24+ days The significance of this variation in clot lysis time is not clear In several young women fibrinolysis was more rapid at the time of menses than during the intermenstrual period However, variation among subjects was great Data on the relationship between fibrinolysis and the menstrual cycle will be reported separately

(2) *The Clot Lysis Time in Patients without Evidence of Hepatic Disease* The clot lysis times of 118 patients on the medical and surgical wards were determined in the manner described. These patients did not have hepatic damage that could be determined clinically or by the usual laboratory tests. The clot lysis times of these patients have been plotted in Fig 1B. In the majority of patients without hepatic damage, the recalcified plasma clot did not dissolve before the third day of incubation at 37°C. Six of these patients had obstructive jaundice of short duration and without evidence of hepatic damage. Ascites due to tuberculous peritonitis was present in two patients.

In eight patients, however, the clot lysis time was two days or less. These patients did not have clear evidence of hepatic damage, as detected by clinical criteria or partial laboratory studies. Seven of these eight patients were males, although 41 per cent of the 118 patients were females. Certain data pertinent to the state of hepatic function in these patients have been tabulated in Table I. One patient with hemophilia had a lysis time of two days on two occasions, the clot lysis times in two other hemophiliacs were 5 and 10+ days respectively. Two patients with severe rheumatoid arthritis had clot lysis times of two days. One of these patients had evidence of hepatic damage. In the other, the clot lysis time three days after the first determination was 10+ days. In a third patient with rheumatoid arthritis, the clot lysis time was six days.

Three patients with rapid lysis times apparently were undergoing some form of intravascular hemolysis. One of these patients had sickle cell disease, another had polycythemia vera with jaundice, and a third had typhoid fever with hemoglobinuria and methemalbuminemia. The latter two patients had evidence of hepatic damage as well. However, in a patient with congenital hemolytic jaundice, another with sickle cell anemia, and a third with acquired hemolytic jaundice of unknown etiology, the clot lysis times were within the normal range. The clot lysis time was rapid in a patient with carcinoma of the esophagus, in another with multiple thromboses, in one with orthostatic hypotension, and a fourth with hypertensive cardiovascular disease.

In one patient with subacute bacterial endocarditis due to *Streptococcus mitis*, the clot lysis time was 6 hours, six hours after embolectomy and while the patient was being treated with dicumarol. Five days

TABLE I\*  
Rapid Lysis Time in Patients Without Hepatic Disease

PT	AGE	SEX	DIAGNOSIS	LYSIS TIME	CF	TT	BILI	II	BSP	ALB	GLOB	ALK PH'ASE	PT	UROBILIN- OGEN	BILURIA
E W	75	M	Chr alcoholism, carcinoma of esophagus	2 (11-19-46)				5	3 9	2 9					
R M	23	M	Hemophilia	5 (12-11-46) 2 (2-14-47) 2 (3-15-47)			2 3/1 3	10					>50%	1 10	
P W	23	M	Subacute bacterial endocarditis 6 hrs post op, on dicumarol	1 (3-16-47) 10+ (3-21-47) 15+ (6-24-47)	Neg Neg	6 2 1 6	1 8/0 8		2 9	2 2			<25%		
J N	25	M	Sickle cell crisis	1 (3-22-47)	Neg	4 9	7 9	50	4 0	2 7	14 3		>65%	1 80	
A G	35	M	Rheumatoid arthri- tis	1 (3-25-47) 10+ (3-28-47) 5 (9-17-47)	++	3 8	0 8	75	4 4	2 9	4 0				
H C	47	M	Orthostatic hypo- tension	1 (8-4-47)	Neg	3 5	<0 8	5	4 6	1 7	5 5				
E R	46	F	Hypertensive car- diovascular dis- ease	2 (2-17-47) 2 (2-19-47)	Neg	9 6							>65%		
S W	64	M	Cerebral accident, multiple throm- boses	2 (4-1-47)			<0 8								

\* The laboratory studies recorded in this and the following tables were performed in the laboratories of the Chemical, Biological and Clinical Microscopy Divisions of the Department of Medicine of the Johns Hopkins Hospital. The cephalin flocculation test (CF) is recorded as neg to ++++. The thymol turbidity test (TT) is reported in arbitrary units (14), in this laboratory, the upper limit of normal is believed to be six units. Bilirubin values (Bili) are recorded in mg per 100 cc of serum, where a fraction is reported, the larger value is total bilirubin and the smaller, direct-reacting bilirubin. Icterus Index (II) is recorded in Meulengracht units the upper limit of normal is 10. Bromsulfalein retention (BSP) is recorded in mg of dye retained per 100 cc of serum 30 minutes after intravenous injection of 5 mg per kilo the upper limit of normal is believed to be 10 mg per 100 cc of serum. Albumin (Alb) and globulin (Glob) values are in grams per 100 cc of serum. The alkaline phosphatase (alk ph'ase) values are recorded in Bodansky units. Prothrombin time (PT) is recorded as percentage of prothrombin compared with a normal plasma using an accurate standardized rabbit brain thromboplastin solution the lower limit of normal is 65%. Urobilinogen is recorded as the greatest dilution of urine to give a positive test by the Wallace Diamond method. Biluria was detected by the foam test.

later and again six months later the clot lysis time was 10+ days. Five other patients under treatment with dicumarol had clot lysis times of three or more days. Macfarlane (3) reported that rapid fibrinolysis,

TABLE II  
*Laenne's Cirrhosis*

NAME	AGE	SEX	LYSIS TIME	C F	T T	BLI	I I	B S P	ALB	GLOB	ALK PH'ASE	P T	URO-BILIN OGEN	BILIURIA	COMMENTS
J V	46 M		1 (11-7-46) 1 (6-20-47)	++ ++ ++	17 4 13 1	3 0 1 8/0 8	9	4 9 2 4 5 3	6 3 9 3 4 5	6 4 6 1	45% >50%				Died, no autopsy
W E	44 M		1 (12-2-46)	Neg	5 3	3 5/1 8		5 0 2 7 4 1	4 7	40%	1 80		Neg		Died, no autopsy
F S	41 M		1 (4-14-47)	++	9 3	35 0/20 2	75		2 3 3 4 10 3	30%	1 40				Died, autopsy
E K	40 M		2 (11-21-46)	Neg	21 3	2 2/1 1		2 6 4 9 3 5							Confirmed at operation
J K	60 M		2 (12-16-46)	+	5 6	2 5/1 6	10		4 0 3 3 15 9	>65%	1 80				Died, autopsy, carcinoma of liver and cirrhosis
J H	56 M		2 (1-31-47)	++	8 8	7 5/4 8	70	4 1 2 8 4 2	7 2	30%	1 80		ft trace		
C F	36 M		2 (2-14-47)	++	8 2		15	2 9 3 3 4 0	4 6	>65%	1 2				
V M	57 F		2 (3-1-47) 4 (6-6-47)	Neg Neg	9 4 9 6	<0 8 <0 8	10 6	2 2 3 1 1 7 4 4 2 5 4 7	3 0 >65% >80%						Improved, but died 10-18-47 of hematemesis, autopsy
R M	40 M		1 (2-17-47)	+++	17 8	7 9/4 4	20	5 2 2 7 3 6 11 0		35%	1 80				Died, autopsy
B P	60 M		2 (5-17-47) 3 (6-7-47)	++ Neg	11 0 5 8	2 3/1 4 1 5/<0 8		2 8 3 4 3 2	8 2	100%	1 80				Improved
E W	65 F		1 (6-13-47)	++++	32 0	4 7/2 7		1 8 3 8 2 1		40%	1 50		Neg		Died, no autopsy

M H	55F	2 (6-19-47) 2 (10-3-47)	++ ++ ++	17 3 15 2	5 2/2 5 2 7/1 2	30 15	5 8 2 4 4 3	1 4 1 1 3 8	6 6 5 8	40% 50%	1 80	2+	Improved
L W	48M	2 (6-20-47)	+	10 4	16 4/10 0		10 4 2 9 3 8	25 6	>50%		1 80		Died, autopsy carcinoma of liver and cirrhosis
A D	44F	1 (7-16-47) 1 (11-11-47)	++ ++ ++	22 6 28 5	15 9/10 8 2 3/1 1	75 25	3 4 2 3 2 3	4 4 2 4 4 2	7 8 7 0	45% 35%	Neg Neg	4+	Improved
W V	61M	1 (9-14-47)	++ ++ ++	14 4	3 2/1 6	15	3 9 2 1 3 7	3 4	25%		1 80		Died, no autopsy
A M	43F	11 (7-8-47)	Neg	6 1	<0 8	9	1 5 5 4 2 4	14 3	>65%		1 40		Biopsy of liver, Paget's disease of bone
A L	51M	3 (7-8-47)	++ ++ ++	11 8	1 4/0 8	10	4 3 2 5 3 1	3 1	65%		1 80		
M To	58M	1 (10-3-47)	++ ++ ++	11 5	2 3/1 4	16	2 8 3 0 3 3	6 7	50%				
E C	33M	1 (10-14-47) 4 (1-2-48)	++ ++ ++	22 0	2 5/1 5	20	3 9 2 3 4 0	7 0	50%		1 20		Improved
B F	61M	1 (10-10-47)	++ ++ ++	13 6	3 0/1 4		2 6 2 3 3 7	6 7	50%				Died, autopsy
M Ty	63M	2 (10-15-47)	++ ++ ++	30 1	1 8/0 8		2 5 1 8 4 0		30%				Died, no autopsy
J T	57M	2 (10-15-47)	++ ++ ++	6 1	2 2/1 0	10	4 7 2 9 3 7	14 1	50%		1 80	Neg	
S H	58F	2 (11-3-47)	++ ++ ++	7 8	<0 8	5	2 0 1 6 2 5	16 2	>50%				
J J	58M	2 (11-14-47)	Neg	15 5		7	4 2 3 4 3 6	7 0					Died, no autopsy
H B	44M	2 (12-1-47)	Neg	10 6	18 8/11 8	100	3 6 2 7 4 7	>50%			Neg		
M S	75F	1 (12-1-47)	++ ++ ++	12 9	5 0/1 5	7 0	7 3 2 2 2 9	11 6	30%		1 10		Died, autopsy
R G	51F	5 (10-21-47)	Neg	7 7	<0 8	4	0 4 4 3 2 6	3 7	>80%		0	Neg	Enlarged liver and esoph varices
G B	35M	2 (4-1-47)	Neg	7 0	1 8/1 1	7 5	2 9 3 3 2 1	3 6	45%		1 80		
R M	39M	2 (1-5-48)	++ ++ ++	16 2	10 4/6 8	70	2 0 3 4 6 3		35%		1 256	4+	

measured by a different technique, was common postoperatively. However, in six other patients studied from 4 to 48 hours postoperatively, the clot lysis time was 4 to 25 days, values comparable to those observed in normal individuals.

In summary, then, the clot lysis time of hospital patients without overt evidence of impaired hepatic function was usually the same as that of presumably normal adults. In an occasional patient, however, rapid fibrinolysis did occur. The significance of this observation is not clear, but it is of note that in several of these patients active hemolysis was taking place at the time that the blood was drawn.

(3) *The Clot Lysis Time in Patients with Hepatic Disease* The clot lysis times of 29 patients in whom the diagnosis of Laennec's cirrhosis was made on clinical or pathologic evidence are recorded in Fig 1C, and the clinical data relative to the hepatic status of these patients, in Table II. Rapid fibrinolysis was an almost constant concomitant of cirrhosis of the liver. It will be recalled that rapid fibrinolysis was first described clinically in atrophic cirrhosis of the liver. On the other hand, in 13 patients with acute hepatitis due to various causes, the clot lysis time was always within normal limits. In only five of these patients was the test performed during the first week of the disease. However, two of these patients were in coma at the time the test was performed (Table III).

In 24 patients, evidence was present of impaired hepatic function during the course of some other disease. The data on these patients are assembled in Fig 1D and Table IV. In 12 of these patients the clot lysis time was one or two days, and in all but three of the remaining patients, three days. Later, tests on several of these patients with rapid clot lysis times were normal. Two of the patients with rapid clot lysis times had undulant fever with hepatomegaly, and two had hyperthyroidism with evidence of transiently impaired hepatic physiology. In three other patients with hyperthyroidism, but without hepatic damage, the clot lysis time was normal.

In six patients with Laennec's cirrhosis, it was possible to follow the clot lysis time as the patient improved clinically. In two of these patients, whose disease was clinically milder than the others, the clot lysis time lengthened during the period of observation. In the other four patients, however, clinical improvement was not accompanied by any change in the clot lysis time.

TABLE III  
*Acute Hepatitis*

PT	AGE	SEX	LYSIS TIME	C P	T T	BLU	I I	B S	ALB	GLOB	ALK PH'ASE	P T	URO BILIN OGEN	BILIRUBIN	COMMENTS
S S	17	F	3 (1-31-47)	++	18 6	5 2/3 1	25	2 2			11 3		Neg		Approx 25th day
O K	29	M	4 (3-20-47)	+++	31 7	95 2/52 4	100		4 0 3 3	5 9	30%		Pos		8th day
H B	21	F	10+ (4-1-47)	+++	18 6		6	1 1							Approx 60th day
E J	22	F	3 (4-4-47)	Neg	10 4	4 1	15		4 9 3 2	3 0	>80%				5th week
G F	41	M	8 (6-9-47)	+++	19 2	20 0/12 9	100		3 6 3 1	9 0	>50%		1 320		10th day, comatose (?homol ser jaundice)
L B	39	F	7 (6-19-47)	+	3 2	6 2	50		3 5 1 9	7 7	>50%				5th day, toxic hepatitis due to ingestion of methyl alcohol and paint remover
L L	34	F	4 (10-9-47)	+++	32	6 4/4 4	50		4 1 4 0	5 9	100%		1 80		4th week, homol ser jaundice
M G	23	F	3 (10-9-47)	+++	31 7	7 0/4 9	75		3 6 3 9	16 0	40%		1 80	Pos	19th day, 3rd recurrence
J L	24	M	4 (10-9-47)	+	4 0	10 4/7 1	50			7 5	>50%		1 20	2+	7th day
R B	4	M	15 (11-9-47)	Neg	7 4	7 8/5 3			3 6 2 5	8 3	50%		1 10		7th day, comatose
E R	62	F	4 (11-11-47)	+++	17 8	13 2/9 4	50			10 7					2nd day (? homol serum jaundice)
P H	24	M	3 (2-11-47)	Neg	6 4	4 3/2 8	7 5		4 6 1 9	10 0			trace	Neg	1st day
D L	25	M	10 (2-26-48)	+++	16 3	6 3/4 9	50		6 2 5 7	2 4	9 0	>50%	1 40		6th day



TABLE IV  
Other Hepatic Pathology

PT	AGE	SEX	DIAGNOSIS	LYSIS TIME	C F	T T	BILI	Y I	B S P	ALB	GLOB	ALK PH/ASE	P T	URO-BILIN- OGEN	BILI- URIA
W S	49	M	Hemochromatosis (biopsy)	7 (7-30-47)	Neg	8 5	1 3/0 8		0 2 4 7 2 9	5 4	>80%	1 10			
R L	39	M	Wilson's disease with hepatosplenomegaly, autopsy	4 (12-3-47)			1 2/1 0		3 9 1 9 4 1						
D J	44	M	Undulant fever with hepatic insufficiency	3 (10-10-46) 2 (11-8-46)	+ Neg	5 1 8 3	<0 8 <0 8		3 1 3 1 3 4 3 2 4 2 3 1	4 1 5 5		1 20			
T P	46	M	Undulant fever with hepatomegaly	4 (7-30-47) 2 (9-3-47) 6 (9-14-47)	+++ +++ +++	25 1 31 0	<0 8		0 5 4 3 3 8	3 4 4 7	>65%				
L H	31	M	Sickle cell anemia with hepatomegaly	3 (8-1-47)	++	17 6	10 8/5 7		19 3 9 3 2						
L M	43	F	Acquired hemolytic jaundice with hepatomegaly	5 (2-14-47)	++	11	3 9	15	2 8 4 3 3 4	2 5	>50%	1 40			
J C	56	M	Hypertensive C V disease with congestive failure and C P C of liver	3 (11-7-46) 2 (11-11-46)	+ +	5 4 8 3	2 8/1 6	5	3 4 2 9	3 5	>80%				
K S	57	F	Hypertensive C V disease and C P C of liver	3 (11-19-46)	Neg	8 3	1 8/1 1		3 5 4 0 2 3	4 6					
T J	34	F	Rh heart disease, C P C of liver	3 (6-19-47)	Neg	16 0	2 0		3 4 4 0 4 6						
L W	46	F	Hypertensive C V disease with C P C of liver	3 (6-10-47)	Neg	8 6	<0 8		2 5 3 8 4 3	4 4					
E A	43	F	Hyperthyroidism and pellagra	1 (2-11-47) 3 (2-19-47)	Neg Neg	6 2 4 9	2 3/1 1 <0 8		2 6 2 2 2 9 1 2 2 1 2 3	9 0 9 0					

H C	61	M	Hypertthyroidism	1 (9-3-47) 4 (9-7-47)	Neg	3 2	1 2/1 1 10 <0 8	5	4 23 7 10 0 2 4 7 3	1 80
A K	40	M	Carcinoma of stomach with metastases to liver	3 (11-15-46)	Neg	10 9 15 8/10 5				
C P	76	M	Carcinoma of stomach, as- cites and jaundice with ? metastases to liver	2 (3-15-47)	Neg	1 0 25 8/17 2 75			4 0 2 9 15 7 >50%	
A J	52	F	Carcinoma, primary site un- known, with metastases to liver and bone	2 (6-10-47)	Neg	5 1	<0 8		2 6 2 8 15 9 <20%	1 10
B G	38	F	Carcinoma, primary site un- known with metastases to liver and lymph nodes	3 (10-23-47)	Neg	19 2	2 0/0 8 10		6 2 2 8 3 8 31 5 40%	1 80
H R	59	F	Diabetes melitus, nodular liver at operation	2 (11-17-46)	Neg	17 9	0 8		0 3 4 7 2 7 >80%	
T M	5	M	Biliary cirrh & congenital absence of bile ducts (bi- opsy)	1 (3-22-47)	+++	1 8	57 0	11 6	3 9 3 3 20 3	+++
Z W	55	M	Pneumococcal pneumonia, chronic alcoholism & hep- atomegaly	3 (4-19-47)	++	17 6	<0 8		0 4 3 3 5 0 4 1 >50%	
A B	40	M	Chronic alcoholism, hepato- megaly	3 (2-26-48)	Neg	4 2	1 4/0 8 20		5 7 >65% 1 40	
G L	59	M	Chronic alcoholism, hepato- megaly	3 (1-12-48)	Neg	12 8	1 4/0 8 10		2 7 5 1 3 4 3 8 >80%	Neg
A W	65	M	Rheumatoid arthritis with hepatomegaly	2 (10-10-47)	++	14 1	1 4/0 8		0 5 3 8 2 8 7 1 >65%	Neg
J L	25	M	Typhoid fever with hepato- megaly, hemolysis and methemalbuminemia	2 (11-6-47)	Neg	11 2	2 2/1 0		3 0 3 5 2 7 4 0	
G B	59	M	Polycythemia vera and hep- atomegaly	2 (3-19-47)	Neg	4 0	5 4/2 7		4 3 2 3 2 3 >80%	Neg

Rapid fibrinolysis, then, was an almost invariable accompaniment of Laennec's cirrhosis of the liver. It was present also in patients who had hepatic damage during the course of some other illness, but was not observed either in acute hepatitis or in obstructive jaundice without hepatic damage.

#### DISCUSSION

The data presented indicate that in normal adults the recalcified plasma clot dissolved only after 3 or more days when it was incubated at 37°C. Moreover, the great majority of hospital patients had clot lysis times of three days or longer. On the other hand, in patients with chronic hepatic disease, particularly Laennec's cirrhosis, the plasma clot usually dissolved within two days, a phenomenon which could be demonstrated repeatedly.

The relationship between hepatic damage and rapid fibrinolysis was first noted by Nolf. He observed that rapid fibrinolysis occurred when Witte peptone was injected into hepatectomized dogs (4). If the liver was only temporarily excluded from the general circulation, restoration of the circulation of the liver would abolish the fibrinolytic effect of Witte peptone (5). If a dog was poisoned with phosphorus, an hepatic toxin, its blood became incoagulable, but when fibrogen was added, a clot occasionally formed which then dissolved rapidly (6). These studies suggest that rapid fibrinolysis might follow hepatic damage. In 1914, Goodpasture (1) showed that this was the case, for the clotted blood of patients with atrophic cirrhosis of the liver dissolved rapidly. This phenomenon never achieved wide popularity as a diagnostic measure, although the data presented suggest that it might be useful in determining whether hepatic damage is present in patients with ascites. It will be remembered that in the present study, two patients with tuberculous peritonitis without evidence of hepatic damage had normal clot lysis times.

Although the clot lysis time was rapid in patients with chronic hepatic disease, it was normal in each of 12 patients with acute hepatitis. This was even true in two patients in coma because of severe infectious hepatitis. One is led to speculate whether the phenomenon of rapid fibrinolysis does not indicate a fundamental difference in the pathologic physiology of acute and chronic hepatic disease, or whether it depends upon the duration of the illness.

Rapid fibrinolysis was also observed in eight patients in whom no evidence of hepatic damage was detected, i.e., about five percent of adults without evidence of hepatic damage had rapid clot lysis times. In so complex a phenomenon, this lack of correlation with hepatic damage in some patients is not surprising. Occasional reports of rapid fibrinolysis under a wide variety of conditions have appeared in the literature from time to time. Thus Macfarlane and his associates (3, 7) described rapid fibrinolysis in patients both pre- and postoperatively, and in patients with ulcerative colitis, rheumatic carditis, nephritis, serum sickness, Raynaud's phenomenon (with cold agglutinins), toxemia of pregnancy, lead poisoning, urticaria, and iritis. Other observers have reported rapid fibrinolysis in sudden death after intravenous mercurial poisoning (8), in toxemia of pregnancy and eclampsia (9, 10), and in normal individuals during menstruation (10), and after exercise or the injection of adrenalin (11). Rapid fibrinolysis has been reported on several occasions in patients with severe shock (12, 13). Thus Tagnon and his associates noted rapid fibrinolysis in 8 of 22 patients with medical shock, it is notable that two of these patients had cirrhosis of the liver with hemorrhage, presumably from esophageal varices. It should be emphasized that the technique for demonstrating fibrinolysis varied from study to study, so that comparisons are difficult.

Macfarlane pointed out that in many of the conditions under which he observed fibrinolysis the pathology has often been said to involve hypersensitive reactions. With this in mind, it is of interest that in three of the patients reported in the present study with one form or other of intravascular hemolysis the clot lysis was rapid. Whether an antigen-antibody reaction played any rôle is not clear.

Mechanisms controlling the rate of fibrinolysis have been discussed elsewhere (15). Briefly, plasma has long been known to possess proteolytic activity capable of digesting fibrin. However, the rate of fibrinolysis could not be related to the proteolytic activity present in plasma. Furthermore, plasma has been known to possess an agent which inhibits the plasma proteolytic enzyme. The rate of fibrinolysis could not be related to the activity of this inhibitory agent. However, the agent in fresh plasma which inhibited proteolytic enzyme was found to be unstable and its period of activity was variable. It was short-lived in the sera of patients whose clots lysed rapidly and long-

lived in those whose clots lysed more slowly The speed of clot lysis, therefore, was dependent upon the stability of an antagonist in plasma to the proteolytic activity of the plasma

#### SUMMARY

(1) The lysis time of recalcified plasma clots incubated at 37°C was determined for 49 normal human adults and 184 patients on the wards of a general hospital

(2) In all of these normal adults, and 93 percent of 118 patients without evidence of hepatic damage, the clot lysis time was three days or longer

(3) In 26 of 29 patients with Laennec's cirrhosis, and in only seven percent of patients without evident liver disease, the clot lysis time was two days or less

(4) In 13 patients with acute hepatitis and six with obstructive jaundice, the clot lysis time was three days or longer

(5) In 24 patients with hepatic damage due to other causes, the clot lysis time was two days or less in 12, and three days in all but three of the remainder

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# SOME MANIFESTATIONS OF REFLEX ACTIVITY IN SPINAL MAN WITH PARTICULAR REFERENCE TO THE OCCURRENCE OF EXTENSOR SPASM

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Although the sequelae attending partial injuries of the spinal cord form a fairly common syndrome in civilian practice, the long-surviving patient with complete severance of the cord has been a rare clinical entity. However, the recent world conflict with its large number of casualties from high-velocity missiles resulted in a moderate number of such subjects. Application of the considerable medical and surgical advances of recent years, together with rapid, efficient evacuation, greatly increased the initial survival rates in these men. Paraplegia Centers established during the war employed new techniques of bladder management, assiduous hospital care, and careful medical and physical rehabilitation. These and other factors combined to increase markedly individual and group longevity as compared with the average life span of patients suffering similar injuries during World War I. Coincidentally, an opportunity was provided for the study of an unprecedented number of men with traumatic lesions of the spinal cord.

A brief preliminary report on the occurrence of extensor spasm in spinal man has been published by the authors (1). It is the purpose of this study to present detailed observations relating to certain involuntary activity patterns exhibited by long-surviving patients with complete severance of the spinal cord. Initial observations were made on these men during the summer of 1946, and subsequent studies were continued to March, 1948.<sup>1</sup> Each patient was subjected to neurological examination at frequent intervals. Marked changes in the reflex activities of different patients did not occur. The neurologic examina-

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<sup>1</sup> The data were completely indexed during the latter months of 1947, at which time the senior author was Chief of Surgery, Paraplegia Service, Cushing V A Hospital, Framingham, Mass

tion detailed in each case history, therefore, is an examination selected as representative of the reflex activities manifested by that patient

Heretofore it has been generally assumed that, after recovery from spinal shock, men with total transection of the cord exhibit only flexor spasms of the lower extremities, if they demonstrate any involuntary movements. This assumption appears to rest solely upon the observations of Head and Riddoch who, in 1917, conducted an intensive study of eight patients with verified anatomic cord transection and numerous other patients suffering from complete "physiologic" interruption of the cord or from partial cord lesions (2, 3). Repeated examinations of their transected patients indicated that progression from the stage of spinal shock to one of heightened reflex activity was characterized by certain highly specific findings. Their conclusions may be summarized as follows. On recovery from spinal shock, movements of the flexor type are the only primary motor reactions observed in cases of complete transection of the spinal cord. Diphasic movements of flexion and extension of the lower limbs resembling locomotion are never observed, and the extension following flexor contraction is due entirely to relaxation of the flexor muscles and to gravity. Extensor thrust is never seen. The tone of the flexor muscles is consistently higher than that of the extensors, and the knee and ankle jerks are mere twitches. In direct contrast to this group, those patients with "physiologic" (quotation marks ours) transection or with partial cord damage demonstrate primary extensor motor reactions, movements of locomotion and extensor thrust are present, the weak knee jerk is superimposed upon a tonic contraction of the quadriceps, and the penis and scrotum represent the areas of optimal excitability for evoking unilateral or bilateral extension of the lower limbs.

It has been inferred quite generally that, by study of the activity of the flexor and extensor reflexes of the lower extremities, it is possible to determine whether the cord has or has not been completely interrupted. In reference to movements of extension, Head and Riddoch stated

"The presence of this postural mode of response depends upon the integrity of certain descending propriospinal paths which link up the centres in the pons and medulla. Should these paths be grossly interfered with, primary extension is no longer possible, although the total damage to the spinal cord may be far from complete."

Since the publication of this classic study of spinal man, the principal criterion used in establishing a diagnosis of total severance of the cord has been the absence of extensor spasm in muscle groups below the level of transection. This criterion has been accepted as an all-or-none rule by many neurologists. Fulton (4, p. 136) maintains that

"Instances of spinal paraplegia are seen with complete loss of sensibility below the level of the lesion, and yet they may have marked extensor spasm if the limbs are manipulated, especially if the leg is elevated from the popliteal space. *This is an unfailing sign of an incompletely divided cord* and may be of value both in diagnosis and prognosis."

Grinker (5, p. 388) similarly states

"Paraplegia-in-extension is found in partial cord lesions. It is produced by trauma and never when the cord is completely transected."

Russell Brain endorses the theory that extensor hypertonia depends upon the integrity of the vestibulospinal tracts and that destruction of the latter results in a dominant flexor reflex. He writes

"After a traumatic lesion, causing immediate and complete severance of the cord, paraplegia-in-extension never occurs, because the vestibulospinal tract is interrupted from the beginning, and as soon as the stage of spinal shock has passed, paraplegia-in-flexion develops" (6, pp. 603-604)

Bing and Haymaker (7, pp. 55-56) state

"If in transverse lesions of the spinal cord a flexor withdrawal reflex is present, one should determine whether or not, after withdrawal, the limb in question extends again. The presence of withdrawal only signifies that the transection is complete. If after the withdrawal the extremity becomes extended, the transection is incomplete."

On the Paraplegia Service of the Cushing Veterans Administration Hospital, we have had the opportunity to study 27 verified cases of complete transection of the spinal cord in white males. The levels of transection ranged from D-2 to D-12. In every instance, transection had occurred two or more years prior to the period of examination. Two of these men showed only flexor spasm, two exhibited approximately equal flexor and extensor spasm, and eighteen showed predominant extensor spasm. In no patient was extensor spasm alone elicited.



Five men were completely flaccid and demonstrated no reflex activity in the musculature innervated below the level of cord section. At the time of writing, all of these men are alive, and most are in good general

TABLE 1

*Type, degree, and duration of spasm in twenty-seven patients with complete severance of the spinal cord*

PATIENT		SEGMENTAL LEVEL OF TRANSEC- TION	TIME LAPSE SINCE INJURY *	DURATION OF SPASMS *	DOMINANT SPASM PATTERN
			days	days	
1	D K	D-2	889	847	Extension 4+, Flexion 2+
2	J Z	D-3	969	949	Extension 4+, Flexion 1+
3	V H	D-3	830	712	Extension 4+, Flexion 2+
4	R S	D-2	995	946	Extension 2+, Flexion (+)
5	A S	D-3	1,225	0	Completely flaccid
6	T Mc	D-4	1,075	985	Extension 4+, Flexion 1+
7	J Dr	D-3	1,040	958	Extension 4+, Flexion 1+
8	J T	D-4	1,098	0	Completely flaccid
9	R B	D-5	1,088	998	Extension 0, Flexion 2+
10	D P	D-5	986	866	Extension 3+, Flexion 1+
11	P P	D-5	971	940	Extension 4+, Flexion 1+
12	T M	D-5	736	0	Completely flaccid
13	M D	D-6	1,211	1,001	Extension 4+, Flexion 2+
14	J B	D-6	1,311	1,056	Extension 3+, Flexion 1+
15	P C	D-6	841	820	Extension 3+, Flexion (+)
16	R P	D-6	1,102	0	Completely flaccid
17	H F	D-7	851	830	Extension 0, Flexion 4+
18	G G	D-8	909	888	Extension 4+, Flexion (+)
19	J D	D-8	864	684	Extension 3+, Flexion 3+
20	J W	D-8	1,100	1,079	Extension 4+, Flexion 2+
21	J V	D-9	1,195	1,015	Extension 3+, Flexion 1+
22	L D	D-9	719	539	Extension 4+, Flexion 2+
23	D L	D-9	1,181	1,121	Extension 4+, Flexion (+)
24	F B	D-10	985	893	Extension 4+, Flexion 2+
25	W M	D-10	1,174	1,144	Extension 3+, Flexion 3+
26	E G	D-7	1,283	0	Completely flaccid
27	W D	D-12	953	773	Extension 3+, Flexion 1+

\* Calculations to Oct 1, 1947

(+) = Flexion movements minimally present

physical condition. The extreme reflex activity manifested by many patients in this series has been permanently abolished by anterior dor-solumbar rhizotomy.

The results of our observations are summarized in Table 1. In each of the following case reports, the sources of the data in the first two paragraphs were the patient and his clinical records, respectively, except for the surgical procedures carried out personally by one of us.

*Case 1, D. K.*, age 36, sustained multiple rifle bullet wounds on April 24, 1945, with immediate loss of sensation and voluntary movement below the level of the nipples. X-rays demonstrated severe comminution of the laminae of D-2. Exploratory laminectomy one month later revealed a gap of 1 cm. in the spinal cord at the level of the second dorsal vertebra.

The first involuntary movements appeared approximately six weeks after injury and consisted of "toe-wiggling." Spasms of the abdomen, back and both lower extremities began a few weeks later and flexor spasm reached its maximal severity within four months after injury. Activity then gradually became primarily extensor in type. The upright position proved to be the most effective stimulus for the production of extensor spasms. No autonomic manifestations associated with changes in skeletal muscle tonus were observed. Erections have been infrequent, usually incomplete, and there has been no evidence of ejaculation.

Neurologic examination, 27 months after transection of the cord, revealed a predominant extensor spasm pattern in the skeletal musculature below the level of the lesion. Generalized extension of the lower extremities was invariably evoked by shifting the patient from a sitting to a supine position. The maneuver produced a response which involved the skeletal musculature below the costal margins in rigid extension that was maintained for three to four seconds. The rigidity<sup>2</sup> was followed by a stage of relaxation occupying one to two seconds. Intermittent squeezing of the thighs elicited prolonged extension of both lower extremities. Light touch applied to either thigh produced extension to a lesser degree. The clasp-knife phenomenon was easily demonstrated. Manipulation of the skin of the abdomen, gluteal and lumbar regions provoked no movements. Rubbing of the scrotal skin evoked mild extensor spasms, but never complete rigidity. Penile manipulation elicited poorly sustained turgor of the shaft, with no discernible muscle movements.

Nociceptive stimulation of the plantar areas resulted in hallux dorsi-flexion, spreading of the toes, and classic triple-flexion of the lower extremities. No crossed responses were noted. Midline perineal stimulation resulted in bilateral triple-flexion.

The deep abdominal reflexes could be elicited in all quadrants. The cremasteric reflexes were absent bilaterally. Knee jerks were 4+ bilaterally with sustained patellar clonus, and the latter was frequently masked by rigid extension of one or

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<sup>2</sup> Here, and elsewhere in this report, the term "rigidity" denotes a skeletal muscle response similar to the "rigid pillar" phenomenon which characterizes the positive supporting reaction.

both extremities The ankle jerks were 3+ bilaterally with poorly sustained clonus Dilatation of the rectal sphincter resulted in no discernible responses The Babinski responses were strongly positive

Case 2, J Z, age 22, sustained multiple machine-gun wounds on February 3, 1945 He became immediately paralyzed from the level of the nipples downward X-rays disclosed compound, comminuted fractures of the second and third dorsal vertebrae, and evidence of fragmentation of the neural canal at that level Exploratory laminectomy on December 4, 1946 revealed a complete anatomic severance of the spinal cord with a gap between the ends

Involuntary movements of the lower extremities were first noted twenty days after injury when "someone pulled the hairs along my right thigh and the leg jumped" Bilateral flexor spasms gradually manifested themselves and within six months had become quite frequent and vigorous It became necessary to tie the patient to his bed as a safety measure Occasional extension spasm occurred, but it was not until 18 months after injury that the strength and frequency of extension became marked Stepping movements were noted and sudden overall rigidity would stiffen him out on the bed or slide him out of his wheel-chair Erections were present since date of injury, but there were no ejaculations

Neurologic examinations two years and two months after injury disclosed that, although extension spasm was not precipitated by plantar stimuli, it became manifest immediately if the thighs were stroked or squeezed, if pressure was applied at the popliteal region, or if the patient was shifted from a sitting to a supine position Rigidity was generalized from the level of the xiphoid distally and sustained approximately five seconds If one lower extremity was elevated by lifting at the popliteal space, initial ipsilateral extension occurred and frequently, after a definite lag, crossed to the opposite extremity Rapid lowering of the straight lower extremity evoked severe ipsilateral extensor stiffening which invariably crossed and resulted in generalized extension Rigidity of the adductor musculature was a prominent feature of this picture

Noxious stimuli to plantar areas elicited dorsiflexion of the four toes with no movements of the hallux, moderate dorsiflexion of the foot, and sharp tugging flexion at the knee and pelvis Crossed phenomena included plantar flexion of the foot and visible tightening of the anterior and posterior thigh musculature The abdominal wall was not involved in the flexion reflex The right lower extremity failed to demonstrate complete triple-flexion, but was considerably stronger in extensor manifestations than was the left

The superficial abdominal reflexes were absent, the deep ones were 2+ in all quadrants The knee jerk was 4+ on the right with sustained patellar clonus and 3+ on the left with frequent masking by extensor rigidity Ankle jerks were 2+ bilaterally with sustained clonus The sign of Babinski was not elicited

Case 3, V H, age 22, was struck by a bullet on June 20, 1945, and experienced immediate sensory and motor paralysis below the level of the nipples X-rays demonstrated irregularities of the 4th and 5th dorsal vertebrae An exploratory

laminectomy performed in May, 1947, revealed complete transection of the cord at D-3 with an extensive gap between the rostral and distal stumps

Involuntary quivering movements of the feet and toes were first noted approximately four months after injury, whenever the soles were lightly touched. Gradually the knees began to twitch, and flexion at knee and hip became noticeable. Ten months after their appearance, spasms had reached their maximum, and exhibited no change during the ensuing months. "Stiffening-out"<sup>3</sup> movements occurred soon after the first extensor movements were noted, and flexion alternated with extension after spasms became severe, though extension has been consistently predominant. Bicycling movements were noted frequently. Spasms have been severe enough during the past year to necessitate lashing the lower extremities to the bed each evening. On maneuvering between bed and wheelchair, the patient was commonly troubled by extension severe enough to cause extreme rigidity of the trunk and lower extremities. Erections began simultaneously with flexor manifestations, were complete, and evoked by any type of penile manipulation. Ejaculation has never occurred.

Frequent neurologic examinations 25 months after injury demonstrated essentially similar spasm patterns. Movements of extension were consistently more marked than those of flexion. The former were evoked by almost any type of manipulation excepting nociceptive stimuli to the plantar or genital zones. They were characteristically precipitated by a change from a sitting to a supine position. Such a stimulus resulted in smooth, powerful extension, converting the trunk and lower extremities into a "rigid pillar." Light stroke along the thigh evoked strong ipsilateral extension, but crossed extensor movements were infrequent. The more rostral the area of stimulation the greater was the tendency for crossed phenomena. Light stroking of the perineum and scrotum initiated bilateral extension. Manipulation of the penile shaft evoked slow and incomplete erection, but no other evidence of activity unless the thighs or scrotum were inadvertently contacted. Any attempt to lift either lower extremity, especially if the force was applied at the popliteal space, resulted in immediate generalized extensor rigidity of the limb with crossed increase in extensor tone and, occasionally, bilateral rigidity.

Nociceptive stimulation of the plantar surfaces resulted in hallux dorsiflexion, spreading of the toes, and triple-flexion of the lower extremities. Midline pinprick of the perineum and external genitalia resulted in bilateral triple flexion, the response being unilateral with slight deviation of the stimulus to either side.

Deep abdominal reflexes were present in all quadrants. Knee jerks were 4+ bilaterally with no patellar clonus. Ankle jerks were 3+ bilaterally with poorly sustained clonus. Cremasteric responses were absent bilaterally. The sign of Babinski could be demonstrated in each lower extremity.

Case 4, R. S., age 22, was injured by a bullet on January 8, 1945. He sustained an immediate paralysis below the level of the nipples. X-rays revealed a com

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<sup>3</sup> Words of the patient

pound fracture of the body and laminae of the second dorsal vertebra. Laminectomy the day after injury disclosed a completely pulped and liquefied cord at the level of the second dorsal vertebra. The liquefied matter was removed, leaving a gap of several cms between the rostral and caudal cord.

Involuntary movements of the toes were first noted five weeks after injury. Within three months, flexor spasms had spread to hips, thighs, and legs. With drawal activity was so constant and severe a month later that surgical relief was advised but the patient refused. Triple-flexion was frequently bilateral at this stage. After one to two months at peak activity, spasms began to decrease in strength and "sensitivity" and, coincidentally, extensor movements appeared and slowly increased in severity. Seven months after injury, flexion had definitely become a minor part of the spasm picture, and extension remained dominant to the present date. The patient has been able to stand without braces or other support for periods as long as one minute before buckling occurs at the hips or knees. He has noted that the act of reclining supine is especially productive of bilateral trunk and lower extremity extension spasms. Erections have been frequent since injury and easily elicitable by manipulation. Ejaculation has been noted on two occasions, accompanied in each instance by extremely severe extensor rigidity.

Neurologic examination 2 years and 8 months after injury revealed a weak, incomplete flexion reflex. The extension pattern was considerably stronger and more easily elicited. Primary extension could be precipitated by lowering the patient from a sitting to a supine position and was manifested by rapid tightening of the trunk, pelvic and lower extremity musculature. Rigidity was not well maintained, however, and relaxation occurred within a few seconds. Rapid lowering of the elevated extremity to the bed resulted in generalized quivering extension of the ipsilateral limb, but crossing was rare, and quite incomplete, when it did occur. Rubbing or squeezing the skin of the thighs elicited no responses. The adductor muscles exhibited moderate hypertonus in the resting state and passive separation of the thighs served to increase adductor tone markedly. Percussion of one adductor tendon resulted consistently in bilateral adductor contraction, drawing the thighs strongly together. Erection occurred readily with gentle penile manipulation, but there was no ejaculation, and no muscle movements.

Noiceptive plantar stimulation provoked weak flexor muscle contractions but did not result in movement of the extremity. There was moderate hallux dorsiflexion, fanning of the remaining toes, dorsiflexion of the foot and visible contraction of the thigh musculature. Noxious stimuli applied to the genital area did not elicit muscle responses.

The superficial abdominal reflexes were 2+ in the upper and absent in the lower quadrants. The deep abdominals were 2+ in all quadrants. The cremasteric reflexes were not elicited. The knee jerks were 4+ with unsustained clonus bilaterally. The ankle jerks were 3+ with unsustained clonus.

Case 5, A S, age 27, sustained multiple machine-gun wounds on May 23, 1944. He experienced immediate sensory and motor paralysis below the level of the

nipples X-rays demonstrated comminuted fractures of the 3rd and 4th dorsal vertebrae Exploratory laminectomy within 48 hours of injury disclosed a completely severed spinal cord at the level of D-3

The patient has never noted muscle movements in any paralyzed part of his body

Neurologic examinations three years and five months after injury revealed a completely flaccid paralysis below the level of the D-3 dermatome No perceptible muscle response occurred to a variety of stimuli Deep and superficial reflexes were not elicited The anal sphincter was patulous

*Case 6, T McC*, age 32, was struck in the chest by a shell fragment on October 20, 1944 There was immediate and complete loss of sensation and motor power below the level of the nipples X-rays revealed comminuted fractures of the laminae of D-5 and D-6 with fractures of the vertebral ends of the right fifth and sixth ribs Exploratory laminectomy on February 14, 1947, disclosed a complete absence of spinal cord substance at the vertebral levels of D-3, D-4, and D-5

Three months after cord transection, while his feet were being washed, the patient noted quivering movements of the toes Gradually, involuntary withdrawal spasms appeared in the lower extremities Six months post-injury, examination revealed flexor spasms rated as 2+, bilateral unsustained patellar clonus, and complete lack of response of the feet or toes to plantar stimulation Extension spasm became manifest at this time and slowly increased in severity Two years after cord transection, stepping and bicycling movements had become quite violent and it was necessary to tie the patient to his bed or wheelchair Primary extensor rigidity was made especially severe by weight-bearing in the upright position, and brace ambulation was impossible Erections were directly associated with spastic activity, occurring more frequently as spasms increased in severity There were no ejaculations

Neurologic examinations two years and five months after cord transection revealed a patient with remarkably strong and varied involuntary movements At times, activity appeared to be spontaneous Reflex activity was self-propagated for periods of many minutes The dominant spasm pattern was extension Plantar stimulation elicited bilateral extensor rigidity, unusual in that the stiffened lower extremities lifted slightly at the hips and traveled slowly from side to side like a horizontal pendulum, the arc gradually decreasing to the midline The tightly apposed extremities then slowly dropped to the bed, where "plastic" relaxation eventually occurred With more intense plantar stroke, the bilateral rigidity was followed almost immediately by a smooth, rapid abduction of the stimulated limb, the latter lifting and swinging outward from its taut hinge at the pelvis After a definite lag, the contralateral extremity followed, maintaining close approximation to its fellow Return to the midline was followed by eventual relaxation Mild plantar pinprick produced similar extensor phenomena and repeated stimuli occasionally established synchronous stepping or bicycling movements Almost any non-noxious contact with the extremity, such as strolling,

squeezing and occasionally merely touch, evoked complex extensor responses. Extensor thrust was powerful and sustained, and the clasp knife phenomenon easily demonstrated in either lower extremity. *Shifting from a sitting to a supine position invariably precipitated extension so severe as to "knock out the breath"* of the patient during the period that rigidity was sustained. Erections were easily evoked by penile manipulation, but no muscle activity occurred. There was no ejaculation.

Strong pinprick of the plantar and genital areas resulted in quick, jerking withdrawal of the ipsilateral limb, but there were no movements of the hallux or toes. Relaxation was occasionally abrupt, but more frequently the triple-flexed extremity waved uncertainly in the air, rotating at the hip for many seconds before sinking slowly on the bed, where extensor rigidity supervened. Midline perineal pinprick produced bilateral lower extremity withdrawal, without movements of the feet. Noxious stimuli to abdomen, thighs or legs evoked no significant responses.

The superficial abdominal reflexes were absent, the deep were 1+ bilaterally, knee jerks were 4+ bilaterally with severe patellar clonus, masked rapidly by extensor rigidity. Ankle jerks were 4+ and clonus was checked by extensor spasm. No toe movements were elicited.

Case 7, J. D., age 34, was struck by shell fragments on November 24, 1944. He immediately lost all sensation and motor power below the level of the nipples. X-rays disclosed fractures of the 3rd and 4th dorsal vertebrae. Laminectomy on November 14, 1947, revealed a complete transection of the spinal cord at the level of D-3.

Two weeks after injury, bilaterally positive Babinski signs were noted. One month later, withdrawal reflexes were obtained by stroking the inner aspects of the thighs. The patient was first cognizant of reflex activity four months after injury when weak flexion movements of the knees occurred. This involuntary activity gradually spread to hips, back and abdomen. Approximately nine months after injury, the left lower extremity began to exhibit extension. At the end of one year, extensor spasms had become completely dominant in both lower extremities, although the right lower extremity still occasionally exhibited flexor spasms. He noted stepping movements frequently, but no bicycling. Extension has frequently "thrown him" from his wheel-chair by lifting his hips forward and stiffening out the trunk and both lower extremities. Erections have been frequent since injury and occur with any non-noxious penile stimuli. There have been no ejaculations.

Neurologic examinations two years and 10 months after injury revealed extremely strong and active extensor spasms, easily provoked by shifting the patient from a sitting to a supine position or by squeezing the anterior thigh musculature. Primary extension occurred immediately, converting the trunk and lower extremities into a rigid framework, the belly hard, hips and knees locked, thighs tightly apposed, and feet pointed in extreme plantar flexion. Rigidity persisted for approximately five seconds and relaxation was smooth and simultaneous in all spastic muscle groups. Pressure beneath the popliteal space did not usually cause extension, but as passive flexion began at the hips there was immediate powerful

resistance, gradual melting of this resistance was accompanied by steady tightening of the ipsilateral hamstrings and adductors, drawing the leg up and adducting the thigh. Passive hyperflexion resulted in sudden relaxation of the thigh musculature and the clasp-knife phenomenon was thus demonstrable. Forceful flexion of the thigh with the leg fully flexed provoked strong extensor thrust at the hip, which ceased immediately with release of pressure. Although extension could be elicited in one lower extremity alone, crossing was frequent and spread of extension was dependent upon the intensity of stimulation. Penile manipulation evoked complete erection within 30 seconds, without muscle movements of trunk or extremities. There was no ejaculation.

Nociceptive stimulation of the plantar areas elicited strong hallux and foot dorsiflexion, followed by the movements of classic triple-flexion.

The superficial abdominal responses were present in all but the left upper quadrant, the deep reflexes were 1+ bilaterally. The knee jerk on the left was 2+ and on the right 3+. All attempts to elicit clonus resulted in primary extension of the limb being manipulated. The ankle jerk was 3+ on the left and 2+ on the right, with no clonus elicitable. The sign of Babinski was present bilaterally.

Case 8, J. T., age 29, was injured on September 27, 1944, by a shell fragment, and experienced complete sensory and motor paralysis below the level of the nipples. X-rays revealed comminuted fractures of the 5th and 6th dorsal vertebrae. Exploratory laminectomy on December 3, 1946, revealed a 3 cm gap between the ends of the spinal cord at the level of injury.

Since the date of injury the patient has noted no movements in the paralyzed part of his body.

Neurological examinations three years after injury revealed a completely flaccid paralysis below the level of the D-5 dermatome. Various maneuvers and stimuli failed to elicit muscle movements of any type. The superficial and deep reflexes were absent below the level of transection, and the anal sphincter was patulous.

Case 9, R. B., age 28, was struck in the chest and spine by a bullet on October 7, 1944. There was immediate sensory and motor paralysis below the level of the nipples. No bony fractures occurred. A second exploratory laminectomy on September 25, 1945 revealed a completely crushed cord at the level of D-5 and apparent conduction of sensation through many fine fibers associated with the dura. The dura and scarred proximal and distal ends of the cord were completely excised, leaving a considerable gap in the spinal cord. There is no indication that this procedure changed the neurologic status of the patient.

Involuntary movements in the abdominal muscles were noted approximately three months following injury. Gradually these weak twitchings increased in severity and spread to the back and lower extremities. Withdrawal spasms were intense within five months of injury and it became necessary to tie him to his bed each evening. Involuntary movements were flexor in type with moderate adduction, and at no time did he note extension. Two weeks following spinal cord excision in September of 1945, weak bilateral lower extremity flexion began and within a month withdrawal spasms reached their old intensity. The abdominal involve-



ment seemed to have decreased in severity, but flexion manifestations were unchanged. There was no stepping or bicycling, but he discovered that stimulation of one sole often caused flexion of the contralateral limb. Despite bilateral obturator nerve section and adductor tenotomy, thigh adduction and scissoring was a frequent occurrence. Withdrawal movements were common even while sitting in a wheelchair. Erections were easily elicited with any non-noxious penile stimulation, but ejaculation was not noted.

Neurologic examinations three years after spinal cord injury revealed severe generalized atrophy of the hips and lower extremities, and chronic flexion contractions of approximately  $25^{\circ}$  at both knees. Extensor muscle masses were practically non-existent, while the hamstrings were palpable as small, firm bands. The right lower extremity demonstrated more activity to a given stimulus than the left. No movements of the hallux were observed with plantar stimulation, but there was moderate dorsiflexion of the remaining toes, strong dorsiflexion of the foot and definite jerking contraction of the thigh flexors. There were no crossed movements even with severe plantar stimulation. On the left, toe movements were absent, but the other responses, though attenuated, were similar to those elicited on the right. Rubbing of the scrotal skin elicited bilateral hamstring contraction, and pinprick at the genital area provoked ipsilateral or bilateral triple-flexion, dependent upon whether the stimulus was midline or to one side. Erections occurred rapidly with penile manipulation and were complete. There was no ejaculation and no muscle movements attended penile stimulation.

The superficial abdominal reflexes were absent, the deep 2+ in each quadrant, the right knee jerk was 2+, the left 1+. No patellar clonus was elicited. Ankle jerks were absent bilaterally. The sign of Babinski was not present.

*Case 10, D P*, age 25, was struck in the upper spine by a bullet on January 17, 1945, and rendered immediately and completely paralyzed from the nipple line downward. X-rays demonstrated a fracture of the laminae and left pedicle of the 5th dorsal vertebra and a bullet partially imbedded in the body of that vertebra. Exploratory laminectomy on August 9, 1946, revealed that the missile had pierced the spinal cord, severing it. Removal of the foreign body left a wide gap between the stumps of the spinal cord.

Approximately four months after injury, jerking movements of the knees were noted. The patient appreciated the fact that his soles were "sensitive," and weak withdrawal movements were experienced. Within three months, bilateral flexion spasms became severe, and nine months after transection, extreme scissoring activity was ameliorated by bilateral obturator and tibial nerve neurotomies. Crossed flexor movements were noted at this period. Sixteen months after injury, triple-flexion began to alternate with brief episodes of extension and bicycling occurred frequently. Gradually, extensor spasms increased in severity and, two years after severance of the cord, were more frequent and powerful than flexion spasms. The most provocative stimulus for extension was a shift from a sitting to a supine position, though the patient noted that the spasms were evoked also by weight-bearing on the soles. The rigidity proved to be of considerable assistance in maintaining

the upright position During especially active periods, vigorous shaking of the rigid lower extremities occurred, usually terminated by forcible passive flexion of the leg at the knee Erections were frequent and sustained, but there were no ejaculations

Neurologic examinations two years and six months after complete severance of the spinal cord demonstrated a moderate extension spasm pattern and weak withdrawal responses in the lower extremities Extension could be precipitated by squeezing the muscles of either thigh or by having the patient recline supine on his bed The extensor rigidity thus provoked was sustained only for three to four seconds before relaxation occurred Crossed reflexes were demonstrated only by postural change or by vigorous manipulation of the limb, and the musculature below the xiphoid participated generally in the rigidity so produced There was mild resistance to upward pressure at the popliteal fossa, but the limb easily became movable and freely mobile at all joints The clasp-knife phenomenon was rather poorly demonstrated Lateral and medial rotation of the relaxed extremity usually evoked ipsilateral extension, but no crossed muscle responses Manipulation of the muscles and skin of the abdomen, hips, and legs elicited no muscle contractions whatsoever There was no extensor thrust The patient could evoke rather prolonged and rigid extension by "arching" his back repeatedly Erections were easily produced by penile manipulation There were no ejaculations

The deep abdominal reflexes were 1+ in all quadrants Knee jerks were 3+ with unsustained clonus, occasionally masked by primary extension of the stimulated limb Ankle jerks were 2+ bilaterally and clonus was not elicited The Babinski reflex was strongly positive in each lower extremity

*Case 11*, P P, age 21, received multiple machine-gun wounds on February 1, 1945, and was immediately and completely paralyzed below the level of the nipples X-rays disclosed a compound, comminuted fracture of the 5th dorsal vertebra Exploratory laminectomy, on February 2, 1945, revealed a compressed but intact cord at that vertebral level On January 29, 1947, prior to an anterior rhizotomy, re-exploration revealed a large syringomyelic cavity in the cord at D-5 The sac was excised, leaving two slightly scarred stumps with a 2 cm gap between them There was no evidence that this procedure in any way altered reflex activity

The first movements noted in the paralyzed extremities began approximately one month after injury and consisted of slight flexion spasms at the knees These gradually increased in severity and a moderately strong withdrawal pattern was present seven months post-injury A tie sheet became necessary during the night Adduction spasms were producing marked scissoring at this time Ten months after injury, extension spasms appeared and gradually assumed dominance over the involuntary flexion movements Triple-flexion occurred infrequently one year after injury, but as the extensor movements decreased slightly during succeeding months, flexor spasms reappeared and stepping and bicycling were often noted Erections were easily elicited with almost any non noxious stimulation of the penis No ejaculations were noted

Neurologic examinations two years after injury and one month after excision of

the syringomyelic sac disclosed a predominant extensor spasm pattern. Extensor rigidity of the trunk and lower extremities was easily elicited by shifting the patient from a sitting to a supine position, by gently rubbing the thigh or by squeezing the thigh musculature near the knee. Allowing the flexed, relaxed limb to slide flat of its own weight produced ipsilateral extension that frequently crossed in the trunk musculature, but infrequently involved the contralateral extremity. Definite resistance to flexion was encountered at both the hip and knee when pressure was applied at the popliteal space, and after forced flexion the clasp-knife phenomenon could be demonstrated in either limb. Rubbing of the skin of the skin of the legs, abdomen, or lumbar areas evoked no movements. Erections were rapidly evoked by penile manipulation and were unaccompanied by muscle movements. There was no ejaculation.

Jerking flexor movements were elicited by noxious stimuli to the plantar areas. Dorsiflexion of the hallux and four toes was followed in rapid succession by foot dorsiflexion, contraction of the thigh flexors and spasm of the ipsilateral lumbar musculature. Crossed phenomena included plantar flexion of the foot and fleeting primary extension of that extremity. Midline penile or perineal stimuli elicited bilateral withdrawal spasms of both lower extremities.

The superficial abdominal reflexes were absent, the deep ones 3+ bilaterally. The cremasteric responses were active. Knee jerks were 4+ bilaterally with sustained clonus unless masked by extensor rigidity. The ankle jerks were 2+ with bilaterally unsustained clonus. The sign of Babinski was strongly positive bilaterally.

*Case 12, T. M.*, age 24, was struck in the spine by a shell fragment on September 24, 1944. He experienced immediate sensory and motor paralysis below the mid-chest. X-rays revealed comminuted fractures of the 7th, 8th, and 9th dorsal vertebrae. Exploratory laminectomy on December 22, 1944, disclosed a complete transection of the cord at the D-7, D-8 vertebral level, with considerable fibrosis and atrophy of the visible caudal cord.

Since date of injury the patient has noted no muscle movements below the level of his lesion.

Neurologic examinations three years after injury disclosed a completely flaccid paralysis below the level of the D-8 dermatome. Various stimuli and maneuvers failed to elicit muscle movements in the paralyzed portion of his body. Superficial and deep reflexes were absent, and the anal sphincter was completely atonic.

*Case 13, M. D.*, age 26, was injured on June 7, 1944, by multiple shell fragments. He immediately lost all sensation and motor power below the mid-chest. X-rays revealed gross fractures of the 6th and 7th dorsal vertebrae. Exploratory laminectomy on July 19, 1946, revealed a complete transection of the spinal cord at the level of the 6th thoracic vertebra.

Week "jerking" movements at the knees were noted approximately seven months after cord transection. They slowly increased in severity until both lower extremities demonstrated moderate withdrawal responses. One year after injury, extensor spasms appeared and gradually became stronger and more frequent.

Bilateral sustained ankle clonus was observed in October, 1945. Stepping occurred frequently, but there were no sustained periods of bicycling. Spasms did not necessitate lashing him to the bed, but occasionally extension was of sufficient strength to lift him bodily from his wheel chair. The erect position strongly stimulated trunk and lower extremity extension and he was then unable to flex at the hips. Frequency of erections was directly related to increased spasm. No ejaculations were noted.

Neurologic examinations three years and four months after injury revealed a dominant extension spasm pattern, provoked especially by the act of reclining from a sitting position. Rubbing the anterior surfaces of the knees or lower thighs produced similar responses, the entire body below the xiphoid transforming into a "rigid pillar." The stiffened lower extremities frequently jerked clonically as they lifted slightly at the hips, the severe thigh adduction obliterating all inter-thigh space and the feet and toes strongly plantar flexed. Rigidity thus provoked was maintained five to six seconds, and was followed by smooth, plastic relaxation over a somewhat shorter interval. Straight-leg raising evoked immediate strong resistance at the hips and, when this was overcome, the clasp knife phenomenon was demonstrable. Rubbing either popliteal region elicited rapid bilateral extension, maintained for the usual period. Unilateral extension was infrequent, for the abdominal and lumbar responses were invariably bilateral. Involvement of the contralateral lower extremity seemed to be directly related to the strength of the stimulus. Erections were easily evoked by manipulation, although no ejaculation occurred. No muscle movements accompanied penile manipulation.

Nociceptive stimuli on the left sole elicited a strong hallux dorsiflexion with the usual abrupt jerking flexor contractions at leg, thigh and pelvis. On the right, all toes demonstrated quivering plantar flexion. No crossing was noted during such stimulation, nor was the abdominal musculature involved in this reflex pattern. Midline perineal pinprick frequently resulted in bilateral withdrawal movements.

Stroke across the left lower abdominal quadrant consistently evoked contractions in the right upper quadrant. Deep abdominal reflexes were 2+ in all quadrants. Knee jerks were 4+ bilaterally. On the left, clonus was masked by extensor rigidity, on the right, clonus was unsustained. Ankle jerks were 4+ bilaterally, with bilateral sustained clonus. The Babinski response was marked on the left, absent on the right.

*Case 14, J. B., age 23, sustained a compression fracture of the 6th and 7th dorsal vertebrae as a result of a parachute jump on February 25, 1944. He developed immediate sensory and motor paralysis from the mid-chest down. Surgery was deferred until December 1944 when a laminectomy revealed a complete transection of the cord at D-6.*

Eight and one-half months following injury the patient noted twitching of both knees. Involuntary movements increased from mild flexion spasms to severe classic triple flexion, and the right lower extremity was noted to be consistently more active than the left. By mid-1945 extensor spasms began to appear, straightening him out rigidly on the bed. There was no bicycling. One year after injury

spasms were so severe that it was necessary to lash the patient to his bed each evening. On a number of occasions extensor rigidity lifted him bodily out of his wheel-chair. Frequency and severity of spasms began to decrease by January, 1946, and reached the present level of activity two years and three months following cord transection. Moderate flexor and extensor spasms are now manifested, and the latter are consistently more severe. Erections have been frequent since date of injury and occur readily with any type of non-nociceptive manipulation of the penis. Ejaculations have not been noted.

Neurologic examinations two and one-half years after injury revealed a dominant extensor spasm pattern. Complete extension was easily produced by shifting the patient from a sitting to a supine position. Generalized rigidity occurred, involving the body from xiphoid to toes, the abdomen becoming board-like, while the pelvis and distal joints locked. When the palm was placed beneath either knee joint and gentle lifting pressure exerted, bilateral primary extension was a frequent result. The knee and hip strongly resisted flexion, but with persistent pressure the clasp-knife phenomenon could be eventually demonstrated. Squeezing of the anterior thigh consistently produced ipsilateral extension and frequently, generalized bilateral extension. Light brushing contact along the posterior thigh evoked the same phenomena. Strong adductor contraction was always an accompaniment of extension.

Nociceptive stimulation at the sole or genital areas evoked triple-flexion movements which were consistently stronger on the left. There was no movement of either hallux during this or other stimulation. Crossed phenomena were never observed to accompany withdrawal responses.

The deep abdominal reflexes were 2+ in each quadrant and the cremasteric reflex strong on the left, but less marked on the right. Knee jerks were 4+ bilaterally. There was sustained clonus on the left, and clonus was masked by extensor rigidity on the right. Ankle jerks were absent bilaterally. The sign of Babinski could not be elicited.

*Case 15, P. C.*, age 23, was struck by shell fragments on January 11, 1945. He immediately lost all sensation and voluntary motor power below the rib cage. X-rays revealed a comminuted fracture of the 6th dorsal vertebra. Laminectomy eight hours after injury disclosed a completely crushed and liquefied cord at that level, with no anatomic continuity between the proximal and distal stumps.

Spasms began approximately three weeks after injury with quivering movements of the toes. A typical withdrawal pattern gradually developed. Neurologic examination two months after laminectomy disclosed 3+ knee jerks, with a Babinski sign and ankle clonus on the right. Mild extension soon was noted, and within two and one-half years of injury, powerful extensor spasm had developed. Violent shaking of the rigidly extended lower extremities occurred frequently. Bicycling was not noted. During succeeding months the vigor and frequency of all involuntary movement gradually decreased, but extensor components retained their relative dominance. Erections have been frequent and easily evocable by manipulation, but ejaculation has not occurred.

Neurologic examinations two years and nine months after injury revealed a patient whose involuntary movements had subsided to the extent that they were not easily elicited by ordinary stimuli. However, both extensor and flexor patterns could be elicited and the predominant muscle contractions were of an extensor type. If the patient elevated his hips by pushing upward beneath his buttocks, primary extension occurred, locking the trunk and lower extremities in rigid extension. Manipulation of the lower extremities could be carried out without perceptible resistance, but straight leg-raising provoked ipsilateral hamstring tightening. The adductors were moderately taut bilaterally and brisk external rotation of the thigh provoked a rigid, trembling ipsilateral extension that was maintained for 2-3 seconds, and then quickly relaxed. Squeezing or rubbing of the thighs elicited no responses. In the standing position the lumbar, pelvic and thigh musculature exhibited marked increase in tone. The erect position was maintained without braces and with only the balancing support of the hands. Any non-noxious contact with the penile shaft elicited rapid, complete erection, and the latter was sustained with stimulation. No ejaculations occurred, and there was no extraneous muscle movement noted during such stimulation.

Nociceptive stimuli to the plantar areas resulted in dorsiflexion of the ipsilateral hallux and foot, and abortive triple-flexion. Crossed movements did not occur.

The superficial abdominal reflexes were absent, the deep abdominal reflexes were 2+. A fleeting left cremasteric response was noted. Knee jerks were 4+ bilaterally with sustained clonus and ankle jerks were 3+ bilaterally with unsustained clonus. The Babinski response was marked bilaterally.

*Case 16*, R. P., age 26, was injured by machine-gun fire on September 13, 1944. He experienced immediate sensory and motor paralysis below the mid-chest. X-rays revealed a comminuted fracture of the body and laminae of the 6th dorsal vertebra. Exploratory laminectomy the day after injury disclosed a complete severance of the cord at the level of the D-6 vertebra, with a sizable gap between the ends.

The patient has not noted movements of the musculature innervated below the level of the lesion since time of injury.

Neurologic examinations three years after cord transection revealed a completely flaccid paralysis below the level of the D-6 dermatome. Severe nociceptive stimuli failed to elicit any perceptible responses. Superficial and deep reflexes were absent, and the rectal sphincter atonic.

*Case 17*, H. F., age 23, was injured on June 1, 1945, by shell fragments which produced an immediate sensory and motor paralysis from the level of the 7th rib downward. X-rays revealed metallic foreign bodies within the spinal canal at the 7th and 8th dorsal vertebrae. Exploratory laminectomy on August 29, 1946, revealed a 4 cm. scar surrounding a large fragment of metal within the spinal canal at the level of the 7th dorsal vertebra. There was a large gap in the spinal cord following removal of both scar and fragment. This procedure produced no apparent change in the neurologic status of the patient.

Twitching movements began in the hips approximately three weeks after cord

injury and gradually spread to the lower extremities. Withdrawal was consistently stronger on the left. Occasional severe adductor spasms occurred. Increase in the frequency and severity of the involuntary movements continued for 16 months, during which time the patient noted occasional "stiffening-out" spasms, but they occurred only under very special circumstances. Flexion deformities of the lower extremities appeared and it was necessary to tie the patient to his bed whenever he occupied it. Erections were first noted four months after injury and recurred frequently as a result of light contact or irritating stimuli to the penis. There were no ejaculations.

Neurologic examinations two years after cord transection disclosed severe symmetrical atrophy of the hips and both lower extremities. The right lower extremity was drawn up in a permanent flexion contracture of 15 degrees at the knee, and there was little substance to the extensor muscle groups bilaterally. Extensor movements could not be elicited. Plantar stroke or pinprick resulted in strong hallux dorsiflexion with marked fanning of the remaining toes, dorsiflexion of the foot, flexion of the leg on the thigh, and flexion with strong adduction of the thigh. The abdominal and lumbar musculature stiffened bilaterally. No other crossed responses were detected. Lowering the patient from a sitting to a supine position precipitated bilateral withdrawal reflexes. Noxious stimuli applied to calves, thighs, or abdomen either elicited no response or, rarely, triple-flexion of one or both lower extremities. Midline penile or perineal pinprick provoked bilateral withdrawal. Erections were easily evoked by penile manipulation, but ejaculation did not occur and no muscle movements were noted.

Superficial abdominal reflexes were absent. The deep abdominals were 2+ bilaterally, knee jerks were 2+ with no clonus. Ankle jerks were 2+ bilaterally and clonus was absent. Strongly positive Babinski signs were present bilaterally.

Case 18, G G, age 24, was struck by shell fragments on April 24, 1945, sustaining fractures of the right pedicle and both laminae of the 8th dorsal vertebra. He experienced immediate sensory and motor paralysis below the level of the rib cage. Laminectomy on April 9 revealed a lacerated dura beneath the laminae of D-8 and a 0.5 inch gap in the spinal cord at the level.

Involuntary movements were noted three weeks after injury when toe and foot twitching appeared on the right side. Spasm gradually spread to involve the flexor muscles and the patient developed bilateral withdrawal responses. Extensor movements began after triple-flexion had become well marked. Gradually extensor spasms assumed dominance. Sudden extensor rigidity would then frequently lift him bodily from his wheelchair, stiffening him out from xiphoid to toes. Scissoring spasms of the thighs occurred frequently in the sitting position.

Neurologic examinations two years and five months after transection of the cord revealed a strong and persistent extensor activity. Almost any maneuver caused immediate generalized extension. If the flexed lower extremity was held by the heel and gently extended passively, a powerful driving thrust developed and the limb stiffened into extension, its partner extending almost as rapidly. Ipsilateral extensor movements could be effectively evoked by the light stroke of a finger

along the thigh. Crossed movements were extremely common and the clasp-knife phenomenon consistently demonstrable. Pressure, and occasionally touch, at the popliteal fossa produced primary extension, making it virtually impossible to lift the extremity until the musculature had been given ample time to relax. Maintenance of generalized extension resulted in gradual penile engorgement, and erection was complete within 30 seconds. Erection could be easily evoked by manipulation, in which case no muscle movements occurred. There was no ejaculation.

Nociceptive stimuli applied to the plantar areas resulted in the muscle contractions characteristic of classic triple-flexion, but these were neither forceful nor sustained, and the stimulated limb often did not lift from its relaxed position.

The superficial abdominal reflexes were absent, the deep ones were present and 2+ bilaterally. The cremasteric reflexes were present and vigorous, but elicitable only from a sharply circumscribed area the size of a silver dollar at the medial aspect of each mid-thigh. Knee jerks were 4+ bilaterally with sustained clonus.

*Case 19, J. D.*, age 25, was injured by machine-gun fire on June 18, 1945. He sustained immediate sensory and motor paralysis below the waist, and X-rays disclosed a bullet within the spinal canal at the level of the 8th dorsal vertebra. An exploratory laminectomy on March 26, 1947, disclosed an anatomically complete transection of the cord with a gap between the ends.

Weak twitching movements at the knees were first noted approximately six weeks after injury. Typical withdrawal spasms gradually manifested themselves, but at three months post-injury, the lower extremities would occasionally straighten out rigidly. He developed alternating flexion and extension spasms, but extension did not become powerful nor did flexion continue to increase in severity. From day to day, either flexion or extension might predominate, and he believed the movements to be of equal intensity. Erections were noted within a few days after injury and priapism has occurred intermittently since, with no evidence of ejaculations.

Neurologic examinations two years after injury revealed approximately equal flexor and extensor manifestations. The right lower extremity exhibited stronger triple-flexion than the left. Extension was provoked by straightening the partially flexed extremity, by shifting the patient from a sitting to a supine position, or by gentle pressure beneath the knee. Primary extension was thus produced, crossed movements being manifested predominantly in the trunk musculature, but also in a consistent contralateral tightening of the adductor muscles. Clonic jerking of the entire limb was succeeded either by abrupt triple-flexion or by extensor rigidity. Bilateral extension was not noted. If one extremity was held in full flexion and the sole of the contralateral extremity stimulated, a complete reversal of pattern occurred, the flexion of the limb stimulated occurring simultaneously with crossed extension. The clasp-knife phenomenon was evokable bilaterally. Penile manipulation led to partial erection. There was no ejaculation. No muscle movements attended penile stimulation.

Withdrawal response to minimal plantar stimuli was vigorous and immediate. Strong hallux dorsiflexion and fanning of the remaining toes was rapidly succeeded



by dorsiflexion of the foot and strong contraction of the ipsilateral hamstrings, adductors, and iliopsoas. If the opposite extremity was in a position of passive flexion at the knee, this reaction-pattern crossed, but if extended, the crossed extension was greatly augmented. Severe nociceptive stimuli to the genital areas only occasionally evoked flexor movements and these were incompletely manifested.

The superficial abdominal reflexes were absent, the deep reflexes were 2+ in all quadrants. Knee jerks were 3+ bilaterally and patellar clonus was frequently masked by extensor rigidity. No cremasteric responses were elicited. The ankle jerks were 3+ bilaterally with sustained clonus. The Babinski response was bilaterally positive.

*Case 20, J W*, age 27, was wounded by shell fragments on September 25, 1944. He sustained immediate and complete paralysis below the waist. X-rays demonstrated comminution of the laminae and pedicles of the 9th dorsal vertebra. Laminectomy October 15, 1946, at the site of a previous unsatisfactory exploration revealed a transected cord with a 1 cm gap between the stumps.

Twitching of the low back muscles was noted approximately three weeks after injury. As the involuntary movements became stronger, activity spread to the anterior abdomen and thence down both lower extremities. A strong withdrawal pattern developed and within seven months of injury, his lower extremities were so active that it was necessary to lash him to his bed in order to prevent falls. Bilateral jack-knifing was quite common, and the right lower extremity consistently exhibited more activity than the left. One year after injury, he was beginning to note extensor spasm and with succeeding months, extension occurred more and more commonly. Flexor spasms simultaneously decreased in vigor and the patient noted that lifting himself from the bed to the wheelchair often precipitated rigid extension. Pelvic extension was a material aid in helping him maintain an erect posture. Flexor activity while sitting in the wheelchair was noted occasionally. Apparently spontaneous erections were complete while the patient still retained a urethral catheter. After its removal they were partial and ejaculations were never noted.

Neurologic examinations two years and four months after injury disclosed a dominant extension spasm pattern. The threshold for production of such activity was sufficiently low so that plantar stroke evoked ipsilateral lower extremity extension with crossed hallux plantar flexion. If the flexed extremity was allowed to slide flat on the bed, primary ipsilateral extension occurred and crossing was quite rapid, resulting in generalized stiffening of the paretic trunk and lower extremities. The extremity originally stimulated exhibited hallux dorsiflexion, while the contralateral hallux was invariably plantar flexed. Lifting beneath the knee consistently evoked ipsilateral extension with crossing and the same overall picture. The clasp-knife phenomenon was well demonstrated bilaterally. Squeezing of the thighs provoked moderate extension that frequently crossed after a definite lag. Partial erection was produced with penile manipulation and was unattended by muscle movements. There was no ejaculation.

Nociceptive stimuli on the plantar surface elicited the movements of triple-

flexion, but much less completely and vigorously than similar stimulation at the upper inner thigh and genital areas. The only crossed response was plantar flexion of the hallux. Flexor spasm was bilateral, when evoked by stimuli at the perineal midline.

The superficial abdominal reflexes were absent in the upper and 2+ in both lower quadrants. The deep abdominals were 3+ in all quadrants. The knee jerks were 4+ bilaterally and clonus was rapidly masked by extension. The ankle jerks were 4+ with bilateral sustained clonus. The sign of Babinski was bilaterally strongly positive.

Case 21, J. V., age 26, was injured on June 23, 1944, by shell fragments which fractured the right pedicle of the D-9 vertebra and fragmented D-11. There was immediate sensory and motor paralysis from the mid-abdomen down. Laminectomy performed on June 30, 1944, revealed a complete transection of the cord with a gap between the ends at the level of the tenth dorsal vertebra.

"Bucking" movements of the pelvis while the patient was in a prone position were noted six months after injury. One year later, when the patient began to ambulate, he noted jerking at the knees, together with strong dorsiflexion of the feet. A moderate withdrawal pattern developed bilaterally. Extensor movements occurred at this time and flexion and extension movements began to alternate. There was frequent "stepping" but never well-propagated bicycling, and the right lower extremity was always more active than the left. Gradually the extensor pattern became dominant and the flexor spasms simultaneously decreased in vigor and frequency. The patient frequently was able to stand without braces, supported by the strong extension which occurred with weight-bearing on the plantar surfaces. Erections were frequent, and were provoked by any non-noxious moving contact with the penis. There was no evidence of ejaculation. The patient has been married the past six months and states that he is capable of maintaining intercourse indefinitely when he so desires.

Neurologic examinations three years and three months after injury disclosed a predominant extensor spasm pattern. Squeezing the muscle masses of either thigh, or lifting the extremity at the popliteal space, evoked primary extension of the ipsilateral limb, and frequently crossed extension. Shifting of the patient from a sitting to a supine position provoked bilateral extensor rigidity without involvement of the abdominal musculature. Strong adductor contraction was part of the extensor pattern. Tapping the adductor tendon would consistently elicit bilateral adductor contraction. Gentle rubbing of the penis provoked rapid and complete erection, sustained as long as the stimulus was continued.

Flexor spasms were easily elicited by nociceptive stimulation of the plantar surfaces, and were characterized by bilateral strong hallux dorsiflexion and classic triple-flexion of moderate severity. These movements were more vigorous and prolonged in the left lower extremity. Elicitation of a withdrawal reflex resulted in contralateral adductor muscle contraction.

The superficial and deep upper abdominal reflexes were present and 2+, the lower abdominals were absent. The cremasteric reflex was not elicited. The

knee jerk was 4+ on the left, patellar clonus could be evoked but was rapidly succeeded by extensor spasm. The knee jerk was 3+ on the right with no clonus. The ankle jerk was 3+ on the right with mild unsustained clonus, 2+ on the left with no clonus. Bilateral Babinski responses were marked.

Case 22, L. D., age 30, was injured on December 21, 1945, when he fell 50 feet from a hangar roof. He sustained immediate, complete sensory and motor paralysis from the mid-waist down. X-rays demonstrated comminuted fractures of the 9th and 10th dorsal vertebrae. Exploratory laminectomy the day after injury disclosed considerable bony and soft tissue damage, and the spinal cord was found completely divided at D-9, D-10, with a gap between the ends.

Involuntary movements were first noted in the feet approximately six months after injury. The lumbar muscles became active and within a few months the patient exhibited bilateral triple-flexion responses. Extension spasms began at this time and slowly increased in severity. Within one year of injury, it became necessary to tie the patient to his bed each evening unless he slept on his side. This peak in the severity of involuntary activity was maintained approximately three months. The spastic manifestations then subsided to a moderate vigor and sensitivity. Bicycling movements were not experienced. Enforced bed rest increased spastic activity markedly. Though spasms frequently interfered with brace walking, the patient believed that the extension occurring with weight-bearing was a distinct postural advantage. Erections began about two months after the first involuntary movements occurred and were easily evoked by manipulation. Ejaculations were not noted.

Neurologic examinations two years after injury disclosed a dominant extensor spasm pattern. The most provocative stimulus for eliciting generalized extensor rigidity was a shift of the patient from a sitting to a supine position. Lowering of the elevated straight leg provoked strong ipsilateral extension that rapidly crossed to involve the contralateral extremity. Squeezing or rotating the relaxed thigh elicited weaker extension responses, usually restricted to the ipsilateral trunk and lower extremity, dependent usually on the vigor with which stimulation was carried out. Bilateral extensor rigidity was frequently interrupted by rapid, clonic flexor movements at the hips, but these intermittent spasms were succeeded by complete rigidity and the latter was maintained 4-6 seconds before plastic relaxation occurred. Rubbing of the thigh surfaces evoked feeble responses or none at all. Lifting beneath the popliteal region infrequently excited ipsilateral extension. Forcible passive hip flexion of the straight lower extremities sometimes evoked weak contralateral extension, sometimes abortive crossed triple-flexor movements. Penile manipulation resulted in rapid erection, sustained with stimulation and unaccompanied by skeletal muscle activity. Ejaculation did not occur.

Nociceptive stimuli applied to the plantar areas resulted in triple-flexor movements of moderate activity. Hallux and foot dorsiflexion were quite marked in comparison to the remainder of the reflex picture. Pin stroke across the left lower abdominal quadrant evoked sharp contraction in the left upper quadrant. The deep abdominal reflexes were 2+ in all quadrants. Knee jerks were 3+ bilaterally.

with unsustained clonus, frequently masked by extensor rigidity. The right ankle jerk was 3+, the left 2+ with bilaterally unsustained ankle clonus. Babinski responses were strongly positive bilaterally.

Case 23, D LaR, age 24, was struck by shrapnel on July 6, 1944. There was immediate sensory and motor paralysis below the level of the rib-cage. X-rays revealed compound, comminuted fractures of the laminae of D-8, D-9, and D-10. Laminectomy on July 12, 1944 disclosed a necrotic and completely severed spinal cord at the level of the D-9 vertebra.

Involuntary twitching movements began in the abdomen and small of the back approximately two months after injury. Muscle activity spread quite rapidly and within a few months vigorous withdrawal reflexes were present. Six months after onset, flexion began to alternate with extension, stepping movements occurred for prolonged periods and the patient was frequently jerked out of bed by sudden severe spasms. Activity was maintained at this high level, although the pattern gradually changed from combined flexion and extension to bilateral extension with weak flexor movements, then reverted to flexion-extension once more. The back muscles were active and quite powerful during early post-injury days, but their activity gradually decreased. Priapism was frequent and could be elicited by manipulation. There was no ejaculation.

Neurologic examinations three years after injury disclosed a strongly dominant extensor pattern. The most effective stimulus for overall trunk and lower extremity rigidity was a shift of the patient from a sitting to a supine position. This commonly resulted in violent, rhythmic shaking of the extended lower extremities, followed by a period of rigidity lasting six to eight seconds. Pressure beneath the knee of either relaxed extremity provoked immediate ipsilateral extension and frequently caused crossed extensor spasm, depending upon the vigor of the maneuver. After extension was forcibly overcome, the clasp-knife phenomenon was consistently demonstrable. Hyper-flexion of the leg with the thigh acutely flexed evoked crossed extension, and forced extension of the leg with the thigh at the same angle elicited crossed triple-flexion. Squeezing of the thigh provoked ipsilateral extension with infrequent crossing. The left lower extremity was slightly more active in flexor and extensor movements than the right. Complete erection was easily produced by penile manipulation, no ejaculation occurred, and no muscle movements accompanied manipulation.

Nociceptive stimuli applied to the plantar areas elicited the jerking movements of classic triple-flexion on the side stimulated. There were no crossed movements. Midline penile and perineal pinprick evoked bilateral triple-flexion, with, however, no movement of feet or toes.

Stroke or pinprick of the left lower abdominal quadrant elicited vigorous contraction in the right upper quadrant. The deep abdominals were present and 2+ bilaterally. The knee jerks were 4+ bilaterally with unsustained clonus. The ankle jerks were 2+ bilaterally with weak, unsustained clonus. The Babinski sign was strongly positive bilaterally.

Case 24, F B, age 34, was injured by shell fragments on January 19, 1945, and

sustained immediate, complete sensory and motor paralysis below the mid-waist. X-rays demonstrated a comminuted fracture of the 10th and 11th dorsal vertebrae. Exploratory laminectomy the day of injury revealed a complete transection of the spinal cord at the level of D-10.

The first involuntary movements of the lower extremities were noted approximately three months after cord transection and consisted of toe "jiggling." Gradually a withdrawal pattern of activity developed bilaterally, at first characterized by jerking movements of the knees, and "bucking" at the hips while lying prone. Six months after injury the triple flexion pattern was moderately active, although it was not necessary to tie the patient to his bed during sleep. Sixteen months after transection, the reflex activity was interfering both with mat exercises and with attempts at ambulation. Extensor spasms began at this time and slowly increased in severity. He found it very difficult to passively flex his strongly extended lower extremities while dressing himself and occasionally extensor rigidity caused him to slide out of his wheel-chair. The act of reclining from a sitting position was invariably productive of extensor spasm. Bicycling and stepping were not noted. The right lower extremity was consistently more active in all reflex manifestations than the left. Erections were present since very early post-injury days, but ejaculation was not noted.

Neurologic examinations two years and four months after cord transection revealed strongly dominant extensor spasms. Primary extension was evoked by a number of maneuvers, including shift of the patient from a sitting to a supine position, upward pressure beneath the knee, and squeezing of the thigh musculature. Crossed movements were frequent, and moderate stimulation resulted in overall muscle rigidity from the mid-abdomen to the toes, the hips locked, the extremities tightly apposed, and feet and toes pointed. When the initial rigidity excited by leg-raising had subsided, the clasp-knife phenomenon was demonstrable at the knee bilaterally. Unilateral extension alone was occasionally elicited by gentle stroking of the skin of the thigh, but if activity was severe, crossed reflexes always occurred. Similar stimuli applied to the skin of the abdomen or legs rarely evoked spasm. Erections were easily produced by penile manipulation, but ejaculation did not occur.

Nociceptive stimulation of the plantar areas resulted in moderately vigorous withdrawal spasm with dorsiflexion of the hallux and foot, and flexion of the leg and thigh. All movements were abrupt in both contraction and relaxation phases. Crossed reflex movements were not noted. Midline perineal or penile pinprick caused bilateral triple-flexion.

The superficial abdominal reflexes were absent, the deep ones were present only in the lower quadrants. There were no cremasteric responses, the knee jerks were 4+ bilaterally, and frequently clonus was masked by extension. The ankle jerks were 3+ bilaterally and clonus was unsustained. The Babinski responses were bilaterally positive.

Case 25, W. M., age 23, was injured July 13, 1944, by a rifle bullet which severely traumatized the left upper arm and eventually lodged within the spinal canal at the

level of the tenth dorsal vertebra. It was necessary to amputate the left upper extremity about 6" below the shoulder. Laminectomy at the site of injury three days later disclosed a "contused" cord. Re-exploration on February 12, 1947, revealed a dense scar within the spinal canal at the level of the 10th dorsal vertebra. This scar was excised, leaving a 2 cm gap between the proximal and distal stumps. Removal of the cicatrix had no apparent effect upon his neurologic status.

Involuntary movements began approximately one month after injury with twitching at the left knee. Shortly thereafter, flexion spasms of the thigh appeared and gradually the movements spread to both lower extremities. Spasms became so powerful that 18 months after injury it was necessary to lash the patient to the bed as a protective measure. Two years after injury, flexor spasms were maximal and it was at this time that extension movements began to appear. As the latter gradually increased in frequency and severity, the flexor pattern slowly subsided and withdrawal manifestations became less powerful. He no longer needed restraint while in bed, and was able to sleep on his abdomen with only occasional "bucking" movements at the hips. Bicycling occurred infrequently. Erections have been frequent, but there has been no ejaculation.

Neurologic examinations three years after injury, and after the scar excision, revealed both flexor and extensor patterns in the lower extremities. Each pattern was of moderate strength and quite easily evocable. The left lower extremity demonstrated sharper, stronger responses in all phases of triple flexion than did the right. Plantar stroke elicited violent dorsiflexion of the hallux and foot, followed by sharp, jerking flexion of leg on thigh and thigh on pelvis. Moderate extensor thrust was elicited, but release of pressure on the sole resulted in relaxation of the limb in the partially flexed position. Noxious plantar stimulation of the right lower extremity elicited only feeble triple flexion, immediately followed by ipsilateral extension and contralateral hallux dorsiflexion, the hallux slowly relaxing as rigidity melted from the stimulated limb. The clasp knife phenomenon could easily be demonstrated on the right. Hyperflexion of the left leg on the thigh produced crossed extension much more frequently than was produced by a similar maneuver of the right leg. Nociceptive stimuli applied to the genital area provoked strong downward contraction of the ipsilateral lower abdominal muscles and a coincident ipsilateral triple-flexion. Midline pinprick of the perineum caused bilateral triple-flexion.

The superficial abdominal reflexes were absent, the deep ones were weak but present in all quadrants. The left knee jerk was 4+ with sustained clonus and the right 3+ with unsustained clonus. The left ankle jerk was 4+ and clonus was sustained. The right ankle jerk was 3+ with unsustained clonus. There was a well-marked Babinski response on the left. Penile manipulation resulted in rapid, complete erection, but ejaculation did not occur. No muscle movements accompanied non-noxious penile stimulation.

Case 26, E G, age 32, was shot in the back on March 26, 1945. He experienced immediate complete sensory and motor paralysis below the level of the rib cage. X-rays revealed comminuted fractures of the 5th and 6th dorsal vertebrae. Ex-

ploratory laminectomy on April 25, 1945 disclosed complete necrosis of the cord at the level of the D-7 vertebra. Aspiration of this material left a wide gap between the ends of the cord.

Since the date of injury the patient has noted no muscle movements in the paralyzed parts of his body.

Neurologic examinations two years and four months after injury revealed a completely flaccid paralysis in the musculature innervated below the D-8 dermatome. The paretic musculature failed to respond to repeated passive manipulation or to various other stimuli. The superficial and deep reflexes were absent below the level of transection, but the anal sphincter was tonic.

Case 27, W D, age 20, sustained a bullet wound of the spine on February 19, 1945. X-rays revealed a large metallic fragment in the body of the 12th dorsal vertebra. Laminectomy of D-11, 12, and L-1 on February 21, 1945, revealed a complete transection of the spinal cord at the level of the 12th dorsal vertebra.

The first involuntary movements were noted in the thighs and knees approximately six weeks after cord transection. Muscle activity spread slowly throughout both lower extremities and bilateral withdrawal patterns had developed eight months post-injury. Occasional extensor movements occurred at this time but were not severe until approximately 16 months after injury, by which time they constituted the dominant spasm pattern. Bicycling movements did not appear, although stepping was noted fairly frequently. Weight-bearing on the plantar surfaces was found consistently to provoke extension of trunk and extremities. The frequency of erections was closely related to the vigor and frequency of reflex muscle activity. There were no ejaculations noted.

Neurologic examinations two years after cord transection revealed a mild but dominant extensor spasm pattern, shifting the patient from a sitting to a supine position was adequate stimulus to evoke generalized extension, involving the lower abdomen, hips and lower extremities. Rubbing the thighs or calves elicited little muscle response, but squeezing the thigh musculature provoked ipsilateral and occasionally bilateral extension. Rigidity was never maintained more than 4-5 seconds. There was definite resistance to flexion of the lower extremity at the knee and pelvis, but once this was overcome, the clasp-knife phenomenon could be demonstrated with either limb. There was no extensor thrust.

Nociceptive stimulation of the plantar areas produced jerking withdrawal movements that were quite attenuated, the involved limb never flexing more than 30 degrees at the hips. There was no crossing noted. Midline perineal pinprick evoked bilateral triple-flexor movements. Strong hallux dorsiflexion was present bilaterally.

The superficial abdominal reflexes were absent, the deep were present and 2+ in the lower quadrants. No cremasterics were elicited. Knee jerks were 3+ bilaterally with unsustained clonus. Ankle jerks were 2+ bilaterally and no clonus was elicited. The Babinski responses were marked bilaterally.

## DISCUSSION

An unparalleled opportunity for the study of long-surviving patients with traumatic lesions of the spinal cord has been afforded by World War II. Knowledge of the reflex functions of the cord has recently been extended and clarified. Thus, earlier views regarding the physiology of the "automatic" bladder have been re-evaluated (8), and hitherto unknown facts relating to the sensory capacities of the isolated spinal cord have been reported (9).

The classic exposition by Head and Riddoch (2, 3) is the only available detailed analysis of the reflex activities exhibited by chronically spinal men. Since none of their subjects were studied later than a year after injury, it is not surprising that our observations, made on men who had survived complete transection for periods of from 24 to 42 months, lead to conclusions regarding the reflex capacities of the isolated human cord which are quite different from those reached by Head and Riddoch. Factors contributing to the present-day high rate of survival and length of life of paraplegics have been mentioned earlier in this paper. It seems certain that most of the apparent discrepancies between our observations and those of earlier investigators can be explained by the greater duration of life and by the better physical condition of our subjects.

In order to obviate any doubts regarding the nature of their lesions, only those men with complete transection of the spinal cord, verified by surgical exploration, were used in the present study. This necessitated the elimination of numerous patients with "physiological" transections, i.e., subjects who had not been explored but were completely lacking in sensation or voluntary movement below the level of injury.

We have made no attempt to categorize our patients as exhibiting "paraplegia-in-flexion" or "paraplegia-in-extension." In the past, these terms, because they have been poorly defined, have resulted in considerable confusion, rather than clarity. Thus, "paraplegia-in-flexion" has been applied in some instances to patients who exhibit predominant, though poorly sustained, flexor movements, and in other cases to subjects with permanent, extreme, cicatricial contractures. "Paraplegia-in-extension" may refer, depending on the particular



author, to the patient with intermittent extensor movements or to one who exhibits more or less continuous and very marked extensor hyper-tonia similar to that seen in acute or chronic mesencephalic and bulbospinal animals (10, 11, 12) In this report, detailed analyses of the flexor and extensor movements elicited in each patient have been presented and no effort has been made to classify rigidly their reflex activity

It is apparent from the case histories (*vide supra*) that, although marked individual variations occur, there is a striking similarity in the general pattern exhibited by most of the patients In our experience, the most typical sequence of events following spinal cord transection in man is 1 Spinal shock, 2 Minimal reflex activity, 3 Flexor spasms, 4 Alternating flexor and extensor spasms, and 5 Predominant extensor spasms The length of time that different subjects remain in a given stage varies considerably In general, stage 1 lasts for one to six weeks, stages 2 and 3, six weeks to one year, stage 4 rarely begins prior to four months after injury, and usually is terminated within one year, and stage 5 may appear as early as six months after injury, and may continue indefinitely Two of our patients did not progress beyond stage 3, and two others remained in stage 4 twenty-four months after transection The majority of the patients were in stage 5 two years after the lesions were incurred It is interesting to note that in all our patients reflex activity first appeared at the distal parts of the limbs and spread rostrally, whereas in recovery from a hemiplegia the proximal parts demonstrate earliest recovery, and spread of activity proceeds distally

It is debatable whether the five patients who failed to exhibit reflex activity of the skeletal musculature below the level of transection were in spinal shock or whether they had suffered not only division of the cord but a lower motor neuron lesion as well Electrical stimulation of the exposed left sciatic nerve of one of our patients (case 5) evoked no response whatsoever (9) On the other hand, an occasional patient may remain in stage 1 or 2 several months, only to progress rapidly through stages 3, 4, and 5 later It is evident, therefore, that the presence or absence of gross reflex activity mediated through the isolated cord is of little prognostic value during the first few months in so far as the eventual recovery from spinal shock is concerned

The cases of Head and Riddoch (2, 3) were strikingly similar to our own before the latter had progressed beyond stage 3. In their studies these investigators make no mention of any observations later than one year after transection. It is of some interest that Dr. Head recorded the following note regarding their patient No. 1, 290 days after injury (2, pp. 295-296)

"On scratching the sole of either foot, flexion occurred at knee and hip, the ankle dorsiflexed, and the toes went up. The flexor movement reached its height after the brief stimulus was removed, and then the leg slowly straightened. *It was unable to end in extensor spasm*" (Italics ours)

This type of reflex response is suggestive of that exhibited by our patients in stage 4.

Many of our patients exhibited reflex activities which are practically identical with those manifested by the chronic spinal dog. Sherrington (13) noted that the after-contraction of extensor muscles in a spinal dog shows a rapid, tremulous decline lasting at longest a few seconds, whereas in decerebrate preparations the reflex movements are of rapid onset but subside sluggishly. At least five of our patients, however, demonstrated generalized extensor rigidity, rapid in onset, sustained fully seven to eight seconds, and succeeded by slow "plastic" relaxation. Powerful, sustained extensor thrust in response to broad innocuous pressure on the plantar surface with the hip and knee partially flexed was not a common finding, but could be elicited in a few patients. Some subjects exhibited strong, sustained thrusting movements at the pelvis when the thigh was forcibly flexed at that joint. Certain of the men with high cord lesions were able to stand without mechanical support for as long as one minute before buckling occurred at the knees. Since under these conditions all muscle groups of the lower extremities were simultaneously in contraction, this demonstrates the occurrence of the positive supporting reaction. Such individuals could stand with ease and for prolonged periods in warm water. These observations indicate that spinal standing can occur in men as well as in the dog.

Ipsilateral withdrawal reflexes were commonly accompanied by crossed extension, and the forced passive extension of one limb often led to crossed triple-flexion movements. Lengthening and shortening reactions were present in all patients exhibiting moderately active

extensor spasms, and the clasp-knife phenomenon was frequently demonstrable. In reference to spinal man, it has been stated that, although the knee and ankle jerks recover, they are easily inhibited, and a clonus never develops (5, 6). Vigorous clonus was frequently noted in our patients, and many were greatly disturbed by the rhythmic "jumping" which often occurred in their lower extremities when their plantar surfaces came in contact with the foot-rest of a wheelchair. When severe extensor activity could be provoked the elicitation of patellar and ankle clonus was commonly succeeded by generalized extensor rigidity of the ipsilateral limb. A few men displayed vigorous patellar reflexes despite the finding of a loose Achilles tendon and an absence of toe responses. The reflex responses of spinal men will be subjected to a more thorough analysis in a forthcoming account. It is relevant, perhaps, to mention that the "mass reflex" and bladder facilitation described by Head and Riddoch were not found in our series of chronic spinal men.

Complete erection could be induced by non-nociceptive stimulation of the penis in the majority of patients manifesting reflex activity below the level of cord injury. Noxious stimulation within the genital area resulted invariably in delayed but progressive detumescence. Indisputable evidence of ejaculation was obtained in one patient (R. S.).

The explanation for the occurrence of primary extensor manifestations in these patients is not clear. In our opinion, such reflex responses probably represent the reappearance of primitive and poorly integrated postural activity mediated by the isolated cord. They stand in sharp contrast to the exclusively flexor responses which characterize the early stages of recovery following cord section and which belong to Sherrington's phylogenetically most primitive group of nociceptive reflexes (14). It appears that the functional capacity of the isolated cord, if given sufficient time to recover, is greater than has been assumed. An analogy may be drawn to the earlier views concerning the capacity of mesencephalic and bulbospinal animals. Recently, many additional righting and postural patterns have been demonstrated in these preparations because for the first time it has been possible to maintain them under satisfactory conditions for long periods (12).

The cases reported in this series are not entirely without precedent

Merritt, Mettler and Putnam (15) mention the occurrence of occasional poorly sustained extensor spasms in patients with complete cord transections. Freeman (16), working with wounded veterans of World War II, has observed instances of extensor spasms exhibited by completely spinal men. Munro, who has probably had more experience with this type of patient than any other recent investigator, does not rely on the presence or absence of reflex activity in the extensor muscles below the level of cord injury, as a criterion of anatomic cord transection (17, 18).

The treatment of spasms of the lower extremities in spinal men deserves mention. Medical therapy has been of little value (19) despite some early encouraging reports on the use of curare. Fortunately, severe spasms of the skeletal musculature can be completely eliminated, in most cases, by anterior rhizotomy (17, 20).

#### SUMMARY AND CONCLUSIONS

1 The involuntary activity patterns of skeletal muscles innervated below the level of lesion in 27 verified cases of complete spinal cord transection have been studied. In each instance, examination was possible at least two years after division of the cord had occurred. Observations were made on men who had survived complete transection of the cord for periods of from 24 to 42 months. The levels of transection ranged from D-2 through D-12.

2 During the periods of observation two patients exhibited flexor spasms alone, two showed approximately equal flexor and extensor spasms, and eighteen displayed predominant extensor spasms. Five patients were completely flaccid below the level of lesion.

3 The most typical progression of reflex activity following spinal cord transection in this series of patients was

- 1 Spinal shock
- 2 Minimal reflex activity
- 3 Flexor spasms
- 4 Alternating flexor and extensor spasms
- 5 Predominant extensor spasms

The length of time that different subjects remain in a given stage varies considerably. In general, stage 1 lasts for one to six weeks, stages 2 and 3, six weeks to one year, stage 4 rarely begins prior to four

months after injury, and usually is terminated within one year, and stage 5 may appear as early as six months post-injury, and may continue indefinitely

4 Extensor thrust is not a common finding but can be elicited occasionally in spinal man

5 "Spinal standing," similar to that seen in animals with transected cords, can occur in spinal man

6 Sustained patellar and ankle clonus frequently occurs in chronic spinal man

7 Primary extensor manifestations in chronic spinal man probably represent the reappearance of primitive and poorly integrated postural activity mediated by the isolated cord. It would appear that the functional capacity of the isolated human spinal cord is greater than has been assumed

8 It is concluded that, though spinal man passes through a period in which flexion reflexes alone are active, he may frequently progress to a stage of activity characterized by predominantly extensor reflexes, amounting in many cases to extensor spasm

9 Extensor spasm in skeletal muscles, innervated below the level of transection, is not conclusive proof of an incomplete division of the human spinal cord

10 It is probable that many of the discrepancies between these observations and those of earlier investigators can be explained by the increased duration of life and, secondarily, by the better physical condition of present-day spinal men

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# PHYSIOLOGICAL STUDIES IN CONGENITAL HEART DISEASE<sup>1</sup>

## VII PULMONARY ARTERIAL HYPERTENSION IN CONGENITAL HEART DISEASE

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Pulmonary arterial hypertension has been observed in patients with several types of congenital cardiac malformations. Dexter and his associates have observed an increase in pulmonary arterial pressure in patients with ventricular septal defect, with auricular septal defect, and with patent ductus arteriosus (1, 2). Brannon, Weens, and Warren have described a case of atrial septal defect in which catheterization revealed marked elevation of the pulmonary arterial pressure (3). In cases of Eisenmenger's complex and isolated septal defects, similar observations have been described in two previous papers of this series (4, 5).

These reports indicate that pulmonary hypertension can occur in various forms of congenital heart disease. In this laboratory, pulmonary hypertension has been frequently observed during the course of study of a large group of patients with congenital cardiac malformations. This paper presents an analysis of these cases, with an attempt to determine the frequency, the distribution, and the dynamics of pulmonary arterial hypertension.

### METHODS

A total of twenty-four patients with pulmonary hypertension, ranging in age from 6 to 46 years, was studied. Only patients in whom the pulmonary artery was catheterized and who had a mean pulmonary arterial pressure of at least 25 mm. of Hg were selected for this report.

<sup>1</sup> Supported by grants from the Commonwealth Fund and the Carolyn Rose Strauss Foundation.

Bloods for analysis from the various parts of the circulatory system were obtained by cardiac catheterization, following the method of Cournand (6) and by puncture of the femoral or brachial artery. Blood gas analyses were carried out in the manometric apparatus of Van Slyke and Neill (7). Arterial and intracardiac pressures were optically recorded by means of a Hamilton manometer (8). In the latter part of this series strain gauge manometers were used. Mean pressures were calculated by planimetric integration of the area under the pressure curve. In four cases (#19, J W, #21, J W, #22, P W, and #24, E F) the mean pressure was calculated from the sphygmomanometric readings by adding one-third of the pulse pressure to the diastolic pressure. Respiratory gases were collected in Douglas bags and analyzed in the Haldane apparatus (9). All gas volumes were expressed in terms of dry gas at standard temperature and pressure (STPD). In the latter part of this series, the Pauling oxygen analyzer was used to determine the partial pressure of oxygen. The standard exercise test used in this study has been described in an earlier communication. Its interpretation and significance will be discussed further in a subsequent publication (10).

The formulae used to estimate the various blood flows were discussed in the first paper of this series (11). In Table I the flows are expressed as indices (liters/min/M<sup>2</sup>). In the calculation of the work of the heart, Table II, uncorrected values were used. For the sake of clarity, formulae used in the calculation of peripheral and pulmonary resistance and in the estimation of the work of the heart are restated.

Pulmonary and systemic peripheral resistances were calculated by Aperia's formula (12)

$$I R = \frac{1332 \times P}{F}$$

where

R = Resistance (dynes/cm<sup>5</sup>/sec), systemic or pulmonary

P = Mean blood pressure (mm of Hg), aorta or pulmonary artery

F = Flow (liters/sec/M<sup>2</sup>), aorta or pulmonary artery

Starling's formulae were used in the estimations of the work of the ventricles (13). Employing these formulae, values are derived for the



velocity and pressure energy developed by each ventricle. In cases of ventricular septal defect, however, particularly when there is *overriding of the aorta*, the presence of the shunt precludes the accurate calculation of these energy expenditures. For this reason, comparison of the work of each ventricle with that of a normal ventricle should not be made. However, certain conclusions may be reached by comparing the relationships of the work of the two ventricles of the same heart. To accomplish this, the ratios of velocity energy to total work of the right and left ventricles are compared. The proportion between the ratio  $\frac{\text{velocity energy}}{\text{total work}}$  (right ventricle) and the ratio  $\frac{\text{velocity energy}}{\text{total work}}$  (left ventricle) may then be compared to that proportion computed for the normal heart. This affords some indication of the work relationship between the ventricles of the abnormal hearts.

II-a Pressure Energy =  $M P \times S D \times D H g$  (Gram-cms)  
where

$M P$  = Mean Blood Pressure in the pulmonary artery or aorta  
(cm of Hg)

$S D$  = Systolic Discharge (cc /beat) =  
$$\frac{\text{Blood flow (cc /min)}}{\text{Pulse rate (beats/min)}}$$

$D H g$  = Density of Mercury (13.6)

II-b Velocity Energy =  $V_a^2 \times S D / 2g$  (gram-cms)  
where

$S D$  = Systolic Discharge (cc /beat)

$g$  = Gravity Constant, 980 cm /sec /sec

$V_a = \frac{S D}{C_a \times E T}$

and

$C_a$  = Cross sectional area of pulmonic or aortic valvular ring  
(cm<sup>2</sup>)

$E T$  = Systolic Ejection Time (sec) =  $\frac{60/\text{pulse rate}}{8/3}$

The cross sectional area of the pulmonary valvular ring was assumed to be  $5.14 \text{ cm}^2$  and of the aortic valvular ring  $3.9 \text{ cm}^2$  (14). In two patients, (#21, J W, and #23, P W), the measurements secured at post-mortem examination of the patients were applied.

The physiological criteria for the diagnosis of ventricular and auricular septal defect and patent ductus arteriosus have been summarized in a previous paper (15). All patients with coarctation of the aorta fulfilled the clinical criteria for this diagnosis (16), which was confirmed at the time of operation. Of the three patients with partial transposition of the great vessels and overriding of the pulmonary artery, the diagnosis was confirmed in one case (#23, P W) at post-mortem examination. The other two patients met the physiological criteria for the diagnosis of this condition (17).

#### RESULTS

Table I lists the data obtained in twenty-four patients with congenital heart disease and pulmonary arterial hypertension. The patients are grouped according to their cardiac malformation. The sequence in each category is determined by the degree of pulmonary hypertension.

Table I shows that thirteen patients had an isolated septal defect, eight of which were ventricular and five auricular. Two patients with patent ductus arteriosus are listed, one with an associated ventricular septal defect. Six patients with coarctation of the aorta are included. Three patients had partial transposition of the great vessels with the aorta arising from the right ventricle and the pulmonary artery overriding the ventricular septum.

The mean pulmonary arterial pressures ranged from 25 to 102 mm of Hg (Table I). In patients with auricular septal defect, the mean pulmonary pressures were from 25 to 82 mm of Hg, while the mean pressure in patients with a ventricular septal defect tended to be higher, ranging from 34 to 102 mm of Hg.

In one patient with a large patent ductus arteriosus (#15, A T), the mean pressure was 35 mm of Hg. In the second patient, where there was an associated ventricular septal defect (#14, H W), the mean pulmonary arterial pressure dropped from 73 to 27 mm of Hg after ligation of the ductus.

TABLE I

*Blood Flows, Blood Pressures, and Vascular Resistance in Pulmonary and Systemic Circulations*

CASE NO	DATE	NAME	SEX	AGE	SURFACE AREA METER <sup>2</sup>	PULM ART PRESSURE		PUL-MO NARY FLOW L/MIN/M <sup>2</sup>		PULMONARY RESISTANCE DYNES/CM <sup>2</sup> /SEC	FEMORAL ART PRESSURE		AORTIC FLOW L/MIN/M <sup>2</sup>		SYSTEMIC RESISTANCE DYNES/CM <sup>2</sup> /SEC	SHUNT L/MIN/M <sup>2</sup>
						S/D mm Hg	Mean mm Hg				S/D mm Hg	Mean mm Hg				
					Normal Range	25/10	18	2 0	720	120/80	93	2 0	3720			
						25/10	18	3 0	480	120/80	93	3 0	2480			
						25/10	18	4 0	360	120/80	93	4 0	1860			
Auricular Septal Defect																
1	2/26/48	J C	F	29	1 52	40/11	25	4 75	423	85/57	70	3 0	1900	1 75		
2	10/28/47	V E	F	27	1 18	49/20	38	2 51	1220	142/87	107	1 65	5200	0 86		
3	2/24/48	J L	M	9	1 17	88/42	62	6 85	726	138/64	75	3 5	1720	5 13		
4	9/15/47	G D	M	16	1 72	74/47	64	2 62	1920	136/88	103	3 33	2490	0 71*		
5	4/2/47	L G	M	30	2 0	103/70	82	1 23	5380	100/73	77	4 78	1230	3 55*		
Ventricular Septal Defect																
6	3/1/48	J G	M	23	1 67	68/9	34	6 35	384	95/83	87	2 78	2500	3 47		
7	9/30/47	A W	M	9	1 16	41/27	34	3 92	696	155/77	106	3 24	2620	0 68		
8	3/30/48	H P	M	23	1 56	75/44	59	1 19	3980	128/75	93	1 97	3800	0 78*		
9	2/3/48	E L	F	29	1 34	92/58	74	1 12	5300	112/62	96	6 6	1160	5 48*		
10	12/12/46	V S	F	25	1 53	112/64	80	2 71	2340	112/79	95	2 5	3040	0 21		
11	3/12/47	B S	M	9	0 92		90	2 28	3130		100	2 72	2960	0 44*		
12	11/15/46	E S	F	19	1 53	122/86	98	2 2	3560	134/82	102	2 62	3120	0 40*		
13	4/11/47	H M	M	24	1 83	115/90	102	1 91	4200	117/103	108	3 86	2230	1 95*		
Patent Ductus Arteriosus																
14	6/16/47	H W	F	16	1 26	97/56	73	2 52	895	111/55	100	5 35	1490	2 83		
	3/22/48	(postoperative)				48/16	27	3 22	670	120/70	84	1 93	2030	1 19		
15	4/13/48	A T	F	26	1 45	43/26	35									
Coarctation of the Aorta																
16	3/10/48	D B	M	22	1 95	40/6	25	3 85	520	134/62	98	3 85	2020			
17	1/22/47	S D	M	45	1 46	44/20	26	2 20	945	137/109	121	2 20	4400			
18	6/26/47	W D	M	17	1 59	44/18	30	2 80	860	171/93	135	2 80	4900			
19	1/27/48	J W	M	15	1 87		35	2 86	980	180/50@	92	2 86	2560			
20	6/20/47	B C	F	20	1 62	49/26	35	8 0	350	200/115	162	8 0	1620			
21	6/24/47	J W	M	6	0 83	116/87	100	2 34	3420	140/90@	102	2 34	3490			
Partial Transposition																
22	2/5/48	H W	M	15	1 41	60/46	49	3 94	1120	129/87	100	2 94	2720			
23	1/28/48	P W	F	5	0 70	57/45	50	2 3	1740	100/80@	87	3 92	1780			
24	5/17/48	E F	M	15	1 35	100/91	95	6 65	1140	100/85@	90					

\* Shunt is from right to left, all other shunts are from left to right

@ Sphygmomanometric pressure

All but one patient with coarctation of the aorta had mild pulmonary arterial hypertension ranging from 25 to 35 mm of Hg. The one patient (#21, J W) with a marked elevation of the mean pressure to 100 mm of Hg also had congenital mitral stenosis and pulmonary arteriosclerosis.

Three patients with partial transposition of the great vessels had mean pressures from 49 to 95 mm of Hg.

The mean systemic blood pressure was elevated in six patients. One patient (#2, V E) had an auricular septal defect. Two patients (#7, A W and #13, H M) had ventricular septal defects. The remainder had hypertension in the upper part of the body associated with coarctation of the aorta.

In three patients with auricular septal defects (#1, J C, #3, J L, and #5, L G), the mean peripheral arterial pressure was below normal, ranging from 70 to 80 mm of Hg. In 14 individuals the systemic blood pressure was within normal limits.

Pulmonary artery blood flow ranged from 1.1 to 8.0 liters/min /M<sup>2</sup> (Table I). Significant elevation of the pulmonary blood flow was found in five patients: one with coarctation of the aorta (#20, B C), two with auricular (#2, J C and #3, J L) and one with a ventricular septal defect (#6, J G), and one with partial transposition of the great vessels (#24, E F). In four patients with isolated septal defects, the pulmonary artery flow was less than 2.0 liters/min /M<sup>2</sup> (#8, H P, #9, E L, #13, H M, and #5, L G).

The aortic blood flow, representing the systemic blood flow plus the collateral circulation to the lungs (11), ranged from 1.65 to 8.0 liters/min /M<sup>2</sup>. The aortic blood flow was elevated in four patients: one with an auricular septal defect (#5, L G), one with a ventricular septal defect (#9, E L), one with a patent ductus arteriosus (#14, H W), and one with coarctation of the aorta (#20, B C). In one patient with patent ductus arteriosus, the aortic blood flow could not be calculated (#15, A T). In this case the oxygen content of pulmonary arterial blood equaled the oxygen content of femoral arterial blood, probably as a result of streamlining in the pulmonary artery. The finding of an unusually large ductus at operation suggested that the aortic flow must have been markedly increased. In three patients with isolated septal defects, the aortic flow was less than 2.0 liters/min /M<sup>2</sup> (#2, V E, #8, H P, and #14, H W).

The left to right intracardiac shunt in patients with isolated septal defects ranged from 0.2 to 5.1 liters/min/M<sup>2</sup>. In the remaining patients with isolated septal defects, the shunt was directed toward the left, ranging from 0.4 to 5.5 liters/min/M<sup>2</sup>. Large intracardiac shunts in both directions were observed in cases of auricular and ventricular septal defect.

TABLE II  
*Results Obtained from the Standard Exercise Test*

CASE NO	DATE	NAME	CC OXYGEN CONSUMED PER LITER VENTILATION	
			Rest	Exercise
2	11/9/47	V E	22	24
4	7/17/47	G D	18	17.5
5	2/4/47	L G	21	46
6		J G	21	21
9	5/22/47	E L	24	22
	2/3/48		25	26
10	12/12/46	V S	21	26
11	2/4/47	B S	24	23.5
12	12/11/46	E S	25	32
13	12/4/47	H M	29	21
	3/4/47		31	24
14	4/24/47	H W	34	29
	6/17/47		24	25
22	2/2/48	H W	21	23
23	1/29/48	P W	17	12
24	3/28/48	E F	18	31

The standard exercise test was performed on thirteen patients (Table II). Oxygen consumed per liter ventilation remained approximately constant or rose during exercise in all but three patients. A repeat test on one patient with patent ductus arteriosus showed no change in oxygen consumption from rest to exercise, (#14, H W). Of the two other patients which showed a drop, one had ventricular septal defect (#13, H M), the other had a partial transposition (#23, P W).

## DISCUSSION

The variations in the degree of the pulmonary and peripheral vascular resistances in the patients of this series are shown in Table I. It may be seen that in seven patients the pulmonary resistance is within normal range. These include two patients with auricular (#1, J C, and #3, J L), three patients with ventricular septal defect (#7, J G, #8, A W, and #14, H W), and two patients with coarctation of the aorta (#16, D B and #20, B C). In all others the pulmonary resistance is elevated, ranging from 860 to 5380 dynes/cm<sup>5</sup>/sec.

The systemic resistance is increased in two patients with coarctation of the aorta (#17, S D and #18, W D), and in one with an auricular septal defect (#2, V E). It should be pointed out, however, that a large part of the total systemic resistance in patients with coarctation of the aorta is probably due to the resistance of the stenosis and collateral blood vessels while the peripheral arteriolar resistance is within normal range (18). In six instances pulmonary resistance exceeds that in the systemic circulation. This group includes a patient with an auricular (#5, L G) and five patients with ventricular septal defect (#8, H P, #9, E L, #11, B S, #12, E S, and #13, H M).

The presence of increased pulmonary vascular resistance is further reflected in the results secured from calculation of the work of the heart

(Table III). The proportion of the ratio  $\frac{\text{velocity energy}}{\text{total work}}$  (right heart)

as compared to this ratio of the left ranges from 0.023/1 to 9.2/1. In the normal the proportion of these ratios is about 3/1. Only one patient, (#6, J G), has a significant elevation of this proportion to 9.2/1. This patient has a large pulmonary flow in the presence of mild hypertension. In ten cases the proportion is within normal range. In the remainder it is markedly reduced, due to an increase in the pulmonary resistance (Table I).

An elevation of the mean pulmonary artery pressure can be produced by one or a combination of three mechanisms. These are: increase in the pulmonary arterial flow in the presence of a fixed pulmonary resistance, second, an increase in the pulmonary vein pressure because of impaired drainage into or through the left side of the heart, and, finally, an increase in the peripheral pulmonary arteriolar resistance.

TABLE III

*The Work of the Heart in Cases with Pulmonary Hypertension*

CASE NO	NAME	RIGHT HEART			LEFT HEART			RT VEL / RT WORK LT VEL / LT WORK
		Velocity energy (gram cm)	Total work (gram cm)	Velocity energy Total work $\times 100$	Velocity energy (gram cm)	Total work (gram cm)	Velocity energy Total work $\times 100$	
Normal*		46	1496	3 1	80 0	7580	1 04	3 0
1	J C	134	2427	5 5	56 0	4056	1 4	3 92
2	V E	13	1943	0 66	6 0	3496	0 17	3 83
3	J L	182	6482	2 8	24 5	3935	0 62	4 5
4	G D	34	3954	0 88	126 0	8136	1 55	0 57
5	L G	7 5	3668	0 21	1050 0	14250	7 4	0 28
6	J G	685	6395	12 0	73 5	5824	1 3	9 2
7	A W	40	2310	1 7	38 0	5838	0 66	2 8
8	H P	3 1	1863	0 17	24 0	4854	0 49	0 38
9	E L	1 2	1429	0 09	442 0	11342	3 9	0 023
10	V S	31	5031	0 61	70 0	6570	1 06	0 58
11	B S	4 3	3064	0 14	13 0	4133	0 30	0 47
12	E S	15	4815	0 31	47 0	6197	0 76	0 41
13	H M	21	6771	0 31	316 0	14716	2 1	0 15
14	H W	17	4467	0 39	286 0	13386	2 2	0 18
	post-op	35	2075	1 7	13 0	3893	0 33	5 1
16	D B	155	2785	5 4	275 0	10275	2 7	2 0
17	S D	13	1203	1 05	22 0	5572	0 39	2 7
18	W D	45	2345	1 9	134 0	10454	1 28	1 47
19	J W	62	2765	2 2	107 0	7207	1 5	1 47
20	B C	575	5965	9 6	1200 0	26100	4 85	1 98
21	J W	7	2087	0 33	14 0	2144	0 67	0 5
22	H W	84	3909	2 2	62 0	5952	1 04	2 11
23	P W	3 9	689	0 55	137 0	2167	6 4	0 086
24	E F	272	11272	2 5				

\* C O = 4500, Rate 75

Mean P A Pressure 18 mm Hg

Mean Aortic Pressure 93 mm Hg

As an example of the first mechanism, elevation of the mean pulmonary pressure in the presence of an abnormally high pulmonary flow, two cases with septal defect may be cited (#1, J C and #6, J G) Both have mild pulmonary arterial hypertension in the presence of large pulmonary blood flow

It seems possible that the increase in mean pulmonary pressure observed in these patients is evidence that the limit of the capacity of the pulmonary bed has been reached Normally, as shown by Hamilton (19) Cournand (20), Riley et al (21), Levy and Blalock (22), and Hickman and Cargill (23), the capacity of the pulmonary vascular bed is sufficient to accommodate a large increase in the circulating blood volume without an elevation of the pulmonary arterial pressure An alternative, that the blood flow exceeds the functional capacity of the left auricle, must also be considered in these cases However, without measurements of the left auricular pressures, the role of this mechanism cannot be evaluated

The second mechanism in the production of elevated pulmonary resistance, an increase in left auricular pressure, is illustrated in patient #21, J W, (Table III) Post-mortem examination in this case revealed a severe degree of congenital mitral stenosis, mild arteriosclerotic changes of the pulmonary vessels, and coarctation of the aorta

The third mechanism causing pulmonary hypertension, an increase in peripheral arterial pulmonary resistance, is illustrated by the findings in several patients As examples, patients #5, L G and #9, E L, (Table I), show high pulmonary pressures, low pulmonary blood flows, and markedly elevated pulmonary resistances In both patients there is present a very large overall right to left shunt (Table I)

The exact nature of the cause of this increased pulmonary resistances cannot be stated Arteriosclerotic changes in the pulmonary vascular bed have been reported in six different congenital malformations of the heart auricular (24) and ventricular (25) septal defects, patent ductus arteriosus proximal to a coarctation of the aorta (26), Eisenmenger's complex (27), partial transposition (17), (case #23, P W) and congenital mitral stenosis with coarctation of the aorta, (case #21 J W) (Table I) It is conceivable that similar anatomical changes are responsible for the increased resistance observed in many cases of congenital heart disease



On the other hand, the possibility must be considered that functional mechanisms may be responsible for a reduction of the cross-sectional area of the pulmonary bed. It is possible that the results of the exercise test afford a means for evaluating the role of these mechanisms. A rise in oxygen consumption per liter of ventilation with exercise may be interpreted as evidence that the pulmonary blood flow can increase sufficiently to take care of the respiratory demands of the standard exercise test. The opinion may be ventured that a rise in oxygen consumed per liter of ventilation, as observed in five of six patients with marked increase in the pulmonary resistance, is more in line with functional rather than anatomical changes in the pulmonary vessels.

#### SUMMARY

The physiological data collected on 24 patients with congenital heart disease with varying degrees of pulmonary hypertension have been presented. The patients include those with auricular and ventricular septal defect, patent ductus arteriosus, coarctation of the aorta, and partial transposition of the great vessels.

Three mechanisms have been proposed which could have produced pulmonary hypertension in these patients:

- 1 Increase in pulmonary blood flow in the presence of fixed pulmonary resistance,
- 2 Increase in the pulmonary vein pressure, and
- 3 Reduction of the cross-sectional area of the pulmonary vascular bed

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## BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

*Brief Psychotherapy* By BERTRAND S FROHMAN 265 pp \$4 00 *Lca & Febiger, Philadelphia, Pennsylvania, 1948*

A book entitled "Brief Psychotherapy" is tempting bait to a physician when offered as an aid in detecting and managing the functional disturbances he finds among his patients. A readable and competent presentation of the clinical aspects of the neuroses has been added to the literature without adding anything to the literature. The reviewer might take exception to various statements and concepts presented, but they are not too damaging. However, the book can be of little help to the practitioner and may well be dangerous. The techniques of "Brief Psychotherapy" described are short-cuts which may be feasible for an experienced therapist. The author, for example, stresses the use of the active analytic technique of Stekel which requires unusual competence in dream interpretation. Even simple dream interpretation is best left to the experienced psychotherapist. Similarly, hypnosis, narcosynthesis, shock therapies, the use of general semantics (Korzybski), have no place in the armamentarium of a practitioner of general medicine. The book has some value as a means of informing physicians how some psychiatrists may go about helping patients, but even here the book may mislead by making some difficult problems sound relatively simple.

T L

*Clinical Diagnosis by Laboratory Methods* 11th ed By JAMES C TODD, ARTHUR H SANFORD, and GEORGE G STILWELL Illus 954 pp \$7 50 *W B Saunders Company, Philadelphia, Pennsylvania, 1948*

This new edition compares favorably with the previous volumes which have made this text a favorite among students and laboratory workers. The illustrations are profuse and excellent. Techniques are clearly presented. The authors have undertaken to cover an almost impossibly comprehensive material in a volume of moderate size. They have succeeded in getting the essence of most of the clinically important methods into the book, but students of the individual sciences may quarrel with the selection, and interpretation of some of the tests. On the whole, however, this is a remarkably successful attempt to compress the whole of laboratory medicine into a readily usable form.

J A L, Jr

*Epithelia of Woman's Reproductive Organs, The* By GEORGE N PAPANICOLAOU, HERBERT F TRAUT, and ANDREW A MARCHETTI Illus 53 pp \$10 00  
*The Commonwealth Fund, New York, New York, 1948*

This short monograph is based on a review of the literature and a complete study of a large series of human organs by the authors. Their discussion of the histology and cyclic changes observed in the ovary, tubes, uterus and cervix compares closely to that given in standard texts of gynecology. However, their thorough study and analysis of the material, with a review of the existing literature, makes the monograph a valuable, complete and concise reference.

The great emphasis on the vaginal cyclic changes and cell smear techniques by Dr Papanicolaou clearly summarizes for the reader the exhaustive work of many years by the master in the field. Most of the many illustrations are in color, large and well chosen. The exceptional excellence of color reproduction and clarity form a major point of praise for this book. It is a beautiful color atlas of the histology of the female reproductive tract during the different physiologic periods.

M B

*Essentials of Public Health* By WILLIAM P SHEPARD, with the collaboration of CHARLES E SMITH, RODNEY R BEARD, and LEON B REYNOLDS Illus 600 pp \$5 00 J B Lippincott Company, Philadelphia, Pennsylvania, 1948

This volume is one of a series of summaries put out by the J B Lippincott Company. It is an excellent and very complete summary of the broad field of Public Health and Preventive Medicine.

The senior author, Dr William P Shepard, has long been associated with the general Public Health movement and has, for many years, participated in the teaching of Public Health and Preventive Medicine at the Stanford University School of Medicine. He is ably assisted by the co-authors, Charles Edward Smith, Rodney Ralph Beard, and Leon Benedict Reynolds, all of whom have had wide experience in various phases of Public Health and Preventive Medicine activities.

The volume was designed to meet the needs, apparently, of teaching of Preventive Medicine and Public Health to medical students. There is, if anything, more than adequate coverage of the traditional environmental control activities dealing with water purification, sewage disposal, and food control, which is almost traditionally dull reading for medical students.

The sections on Communicable Disease Control and Public Health Activities are unusually complete and well written. These sections, particularly, emphasize the role of the practicing physician in the prevention of disease. Factual material contained in the text is accurate and well presented. The frequent use of charts and tables simplifies the reader's understanding of the material.

The medical students and the busy practicing physician should find the volume a handy, brief reference instrument, and the bibliography of each section is sufficiently complete to give him source material for further study. This volume might well be on the shelf of every practicing physician.

E L S

*Hemolysis and Related Phenomena* By ERIC Ponder Illus 398 pp \$10 00  
Grune & Stratton, Inc , New York 16, New York, 1948

Few biological systems have been subjected to so intensive investigation by so many techniques, and by workers in such a wide variety of diverse fields, as the mammalian erythrocyte. The apparent simplicity of this cell, and the ease with which it can be manipulated and observed in experimental studies, have attracted the interest of many laboratories during the past five decades. In "Hemolysis and Related Phenomena", Ponder has brought together much of the accumulated information concerning the red cell, emphasizing throughout his presentation the extraordinary complexity of this apparently simple structure, and the problems regarding its nature which remain unsolved.

The book would seem at first consideration to be of interest primarily to those engaged in the specialty of Hematology. But this is certainly less than the case. The methods and observations described in this compendium will be of interest to workers in many other fields bearing upon the general problem of cellular form and function. The student and physician will use the book as an authoritative reference work for questions which arise in connection with red cell disease, and even though many of the answers will seem theoretical and hardly applicable to practice, they are at least answers. And the general reader, whatever his field, will find considerable pleasure in the reading of this book. The language of Ponder is precise, elegant, and often delightful, and throughout the book one is aware of the well tempered alloy of enthusiasm and skepticism on which it is based.

The book is literally packed with facts, many of which are attended by excellent charts and figures, and there are over five hundred references to primary sources. Chapter I consists of an introductory essay, summarizing the aims and scope of the monograph. Chapters II and III deal with the shape of the red cell, and with the factors involved in changes in shape, particularly with regard to the disc-sphere transformation. Chapter IV is concerned with the cytochemistry and architecture of the cell, and here are discussed the constituents of the red cell, the composition and arrangement of surface structures, and the probable mosaic complexity of supporting tissue and entrapped material which comprise the interior of the cell. The sickling phenomenon is described in considerable detail. Chapters V, VI, and VII deal with the kinetics of hemolysis and the numerous factors which influence hemolysis. The mechanisms which may be implicated in the *in vivo* destruction of the red cells are taken up in the last chapter, and the possible role played by these mechanisms in certain hemolytic diseases is discussed.

There are four brief appendices, dealing with the red cells of the *Camelidae*, the nucleated red cells of the lower vertebrates, the technic of red cell counting, and the metabolism of red cells. The latter appendix contains a great deal of valuable material in concentrated form, and this reviewer would be glad to see it amplified in a future edition and elevated to the status of a chapter.

Lest it be thought that the scope of this book is restricted to a single subject with which it deals, or that the author's preoccupation with this subject is an instance of extreme specialization, it is well to reproduce the following from the

author's introduction "I have been told that I tend to speak of the red cell as if it were a microcosm, and as if an understanding of its nature and properties would include an understanding of nearly everything else in the cellular world To some extent this is true, for there is scarcely a fundamental problem in General Physiology which does not have a relation, of one kind or another, to the problems which have arisen in connection with the erythrocyte" A book which embarks with such a viewpoint, and is at the same time the work of a selective and meticulous mind, is likely to be a good book indeed This one is

L T

*History of the American Medical Association 1847-1947* By MORRIS FISHBEIN  
 Illus 1226 pp \$10 00 W B Saunders Company, Philadelphia, Pennsylvania, 1947

In 1947 the American Medical Association celebrated its hundredth anniversary It was on this occasion that the Board of Trustees of the Association offered the present volume "as a record of its founder, its founding, its ideals and its motivations and the extent to which these have been accomplished over a period of one hundred years" (p v)

It may not be without interest to compare the primary motivations which led to the founding of the American Medical Association and that of its older cousin, the British Medical Association The latter came into existence in 1832 as the "Provincial Medical and Surgical Association" (it assumed its present name in 1856 only) upon the initiative of Doctor (later Sir) Charles Hastings He conceived of its chief goal as "the diffusion and increase of Medical Knowledge in every department of science and practice"<sup>1</sup> Only as a final point did the original prospectus list "Maintenance of the Honour and Respectability of the Profession generally, in the Provinces" etc<sup>2</sup> However, the question of medical education and licensure soon became of great importance and from the late thirties engaged the active interest of the Association<sup>3</sup> Now when Nathan Smith Davis in 1845 suggested the convocation of a National Medical Convention in this country, it was just "the elevation of the standard of medical education in the United States" that was the prime object<sup>4</sup> When the organizational meeting was held in 1846, it took up this desideratum, but also enlarged it by the demand for a uniform code of medical ethics and by appointing committees which were to branch out into more scientific aspects<sup>5</sup> On this basis, the A M A came into life in 1847 The anarchic state of the medical profession in this country as well as in Great Britain, and the stimulus of an age of developing science led the medical associations of the two countries to pursue a similar direction from different starting points

<sup>1</sup> *History of the British Medical Association 1832-1932*, compiled by Ernest Muirhead Little, London, British Medical Association, n d, p 19

<sup>2</sup> *Ibid*, p 21

<sup>3</sup> *Ibid*, p 61

<sup>4</sup> Fishbein, *op cit*, p 22

<sup>5</sup> *Ibid*, p 25 f

Since 1847 some of the main goals for which the A M A had been striving have been reached, but many new tasks have been imposed and new problems, no less controversial than those of a hundred years ago, have to be faced. Dr Fishbein's chronological account, the biographies of the presidents, the sketches outlining the activities of the various councils, bureaus, sections, etc., and of the organs of publication give a picture of the past and present of what is probably the largest medical organization in the world. This picture is not only instructive, but because of the official character of the book it also represents a record that will have to be consulted by all those interested in their profession.

O T

*Hospital Trends and Developments, 1940-1946* By ARTHUR C BACKMEYER and GERHARD HARTMAN 819 pp \$5.50 *The Commonwealth Fund, New York, New York, 1948*

In their earlier work, "The Hospital in Modern Society", the editors covered hospital literature up to 1940. The 148 papers in their present volume represent their choice of the best papers between 1940 and 1946 and cover a wide variety of subjects of importance to both hospital administrator and physician. A comparatively small number of the papers are drawn from the study of more than one hospital, and one can see how desirable studies comparing the methods of one with those of another would be.

C W C

*Intracranial Tumors* 2nd ed By PERCIVAL BAILEY Illus 478 pp \$10.50  
Charles C Thomas, Springfield, Illinois, 1948

The second edition of this classic on brain tumors should be received as enthusiastically as the first edition. A few alterations have been made in the text of this edition to incorporate recent advances in the neurological sciences, but the main thesis, based on the pathological aspects of intracranial tumors, remains unchanged. A series of plates illustrating roentgenographic findings is a welcome addition to the book.

Not only students but general practitioners will find this a valuable, instructive and entertaining monograph on the triumphs and limitations of neurological surgery in a field which has advanced so rapidly in the past two decades.

A E W

*Manual of Clinical Therapeutics, A*, 2nd ed By WINDSOR C CUTTING Illus 712 pp \$5.00 W B Saunders Company, Philadelphia, Pennsylvania, 1948

This book is exactly what the title states—practical therapeutics in compact form. The first two chapters deal with general problems in therapy, and the next eleven with the treatment of various infections. The remainder is divided according to disease categories, first of a general character such as nutritional and endocrine diseases, and then of diseases of the specific systems. Finally there are



nine appendices full of useful information on bedside procedures, physiotherapy, diets and so on

The author intends the book for use by those who may be confronted by therapeutic problems unfamiliar to them. He fulfills this purpose admirably, and throughout the recommended therapies are quite in line with modern formulations. The only criticism is directed not at the content but at the form. A practical manual such as this is divorced from concepts of disease pathogenesis and of the fate of drugs in the body. It is only by means of an accurate analysis of these factors that the therapist will be able to treat the complex situations so often found at the bedside. This book therefore is recommended to those who wish to know how to treat a patient rather than why such a treatment is necessary.

C G Z

*Pathology of Nutritional Disease, The* By RICHARD FOLLIS, JR. Illus 291 pp \$6.75 Charles C Thomas, Springfield, Illinois, 1947

This book is just what it purports to be, a compendium of available information on the pathological anatomy of experimentally induced deficiency diseases. The subject matter is divided into sections on (1) deficiencies of essential elements such as calcium, phosphorus, potassium, magnesium, copper, etc., (2) the essential amino acids, (3) fat and water soluble vitamins, and (4) the essential fatty acids. In the general discussion and summing up of the dietary deficiencies experimentally induced, the author is at pains to emphasize that the facts submitted cannot be directly translated to clinical problems.

The subject matter is clearly treated by the author who has long been active in the field and a prominent contributor. Especially excellent are the treatises on the pathology of rickets and scurvy.

J E H

*Textbook of Bacteriology* 9th ed By HANS ZINSSER Revised by DAVID T SMITH, DONALD S MARTIN, ET AL Illus 992 pp \$10.00 Appleton-Century-Crofts, Inc., New York 1, New York, 1948

This ninth edition of the "Textbook of Bacteriology", which in more recent editions had been written by Zinsser and Stanhope Bayne-Jones, is a standard reference and teaching text in many medical schools throughout the country. Now it has been thoroughly rewritten and revised by Drs David T Smith and Donald Martin and associates at Duke University Medical School. A great deal of new material has been added, and in many cases the most recent references are listed and the work discussed. A large number of electron micrographs is a new and welcome feature in the section on viruses. However, many of the old illustrations should be done over because of poor reproduction in the present edition.

A number of annoying slips or omissions have been noticed throughout the virus section by this reviewer. Panum's classic account of the spread of measles in an immune population does not refer to the South Sea Islands but to the Faroe

Islands, north of Europe The production of encephalomyelitis similar to that following vaccination, by the use of Freund's adjuvant technique, is not mentioned The discussion of the "interference phenomenon" completely ignores the basic work of Magrassi and Hallauer which was done and fully published before 1940 The complete separation of the virus hepatitis complex into (1) epidemic hepatitis, and (2) homologous serum jaundice, a disease "discovered during World War II", is not warranted by the available facts Even those who propose some strain differences as basic to the different methods of spread say only "There are probably several forms of viral infections of the liver which are variants of one general group"<sup>1</sup>

In general, this edition represents a much needed but an imperfect revision of a standard textbook

F B B

*Textbook of Gynecology* 3rd ed By EMIL NOVAK Illus 742 pp \$8 00  
*Williams & Wilkins Company, Baltimore, Maryland, 1948*

This textbook, which includes the fundamentals of gynecology and female urology, stresses the pathological and endocrinological aspects of the subject The author has omitted descriptions of operative procedures and has concentrated his efforts on the diagnostic procedures and medical treatment of gynecological disorders The author has added many new and excellent photographs and photomicrographs in the present edition Ever changing endocrine concepts and therapy have also been brought up to date

J H L

*Topics in Physical Chemistry* By WILLIAM MANSFIELD CLARK Illus 738 pp  
 \$10 00 *The Williams & Wilkins Co, Baltimore, Maryland, 1948*

This is a superb book, unique in its combination of conciseness and breadth of coverage, brimming with useful information in a usable form The reviewer's only criticism is of the title, which is much too vague and dry for this lively opus, and which through fear and prejudice may discourage some medical readers from venturing into this fascinating exposition of what makes the body (and a lot of the doctor's instruments) tick

There is an unusually complete coverage of the principles of chemistry and physics applicable to biological systems and to measuring devices used by the biological scientist The explanations move along briskly, with a minimum of mathematical derivation, but just enough for clarity Limiting laws are presented as such, avoiding the frustration of finding at the end of the chapter that the results cannot be applied to existing systems There is no pomposity of presentation, as the author, with characteristic dry humor, points out the frailties of the "laws" or their misapplications

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<sup>1</sup> Havens, W P and Paul, J R in Rivers' "Viral and Rickettsial Infections of Man", 1948

Each topic is chosen not only for its intrinsic value but for its applicability, and the theoretical expositions are heavily outweighed by the mass of useful applications. Every type of instrument in conventional clinical and research use in biochemical laboratories is considered, briefly but thoughtfully, in terms of what makes it work, how to use it, and what it can be expected to do. Although the application of physical chemical principles to the complex systems of the body is often difficult, there are repeated and well-chosen illustrations of each principle, without visible signs of strain of either the law or the biological facts.

This book can be strongly recommended to every student of the biological and medical sciences as a stimulating and authoritative text or reference source.

J A L, Jr

*Tuberculosis* By FRANCIS M POTTENGER Illus 597 pp \$12 00 C V  
Mosby Company, St Louis, Missouri, 1948

This book is an important contribution to the study of tuberculosis, for it is based on the author's wide experience with the disease over a period of nearly fifty years. The careful compilation of data on the patients in the sanatorium whose progress is followed over a period of years justifies Dr Pottenger's optimistic viewpoint, as he demonstrates the remarkable tendency of the disease to heal spontaneously if diagnosed early and handled well. The conservative therapy which aids in this healing is aimed at building up the patient's resistance from every standpoint while the reactions of immunity take place.

In the chapter on the pathogenesis of tuberculosis the author's discussion does not seem entirely clear in regard to immunity, hypersensitivity, etc. The latter is considered an important part of the protective mechanism, but the evidence presented in support of this belief does not seem adequate for proof, and the difficult problem of the relationship between specific immunity and hypersensitivity in the clinical picture of tuberculosis is not very clearly defined.

The anatomical variations in the thorax at different ages are described and correlated with the tendency for the site of reinfection to shift to the apex, mainly on the basis of a local reduction of blood supply. The importance of this factor is based primarily on two presumptions—that the bacilli are carried to the site of reinfection by the blood stream, and that their source is endogenous rather than exogenous. While either of these presumptions may be correct, neither of them is easily proven and the author's reasons for favoring the "endogenous" theory are not entirely convincing.

Much space is devoted to a detailed consideration of the symptomatic and structural changes produced by pulmonary tuberculosis on various other systems of the body through the mediation of nerve reflexes. Many of these effects he feels are of importance from a diagnostic standpoint, as he finds he can suspect and locate tuberculous lesions in the lungs by observation and palpation of localized alterations in the overlying muscles. While it is interesting to know that these numerous "reflex" effects may be found in pulmonary tuberculosis, one wonders

if their importance in the pathogenesis and diagnosis of the disease may not be unduly emphasized

The indications for the various forms of collapse therapy are outlined, with pertinent discussion of their limitations and dangers. Tuberculin is advocated as an additional therapeutic aid in pulmonary tuberculosis—a view that may be questioned by many other students of the disease. At the end there is an excellent summary of the present status of the use of B C G Vaccine, which Dr. Pottenger shows is proving to be both safe and effective.

The book on the whole contains much valuable information on pulmonary tuberculosis, and there are many well-reproduced photographs of chest X-rays to illustrate the points that are made. Much of it seems repetitious, however, and several of the chapters could be shortened drastically without detracting too much from its fundamental value.

A M F

*Vascular Disorders of the Limbs* 2nd ed. By SIR THOMAS LEWIS 118 pp  
\$2.25 The Macmillan Company, New York, New York, 1946

In a short monograph of 118 pages entitled "Vascular Disorders of The Limbs," Sir Thomas Lewis has sought to bring up to date advances made especially during the ten years preceding the late war relative to disorders of the circulation of the limbs.

The work is written for students and practitioners, so description has been confined to methods of diagnosis which they can employ themselves. At the same time enough is incorporated to insure recognition and adequate management of the different conditions.

The first chapter is called "The Function and Control of the Circulation and Its Testing." Lewis states that three kinds of tests are used in examining the vessels of a limb according to whether they explore (1) the rate of blood flow, (2) the state of various vessels and especially their capacity to dilate, and (3) the intactness or otherwise of the vasoconstrictor nerves.

The description and interpretation of the varieties of skin color add much to a subject so commonly misunderstood.

In commenting on circulatory arrest, the statement is made that the death of muscle fibers is probably assured when in the warm limb they are deprived of blood from six to eight hours, and their death results in a replacement fibrosis, that nerves recover after being ischemic for twelve or even twenty hours, that blistering of the skin occurs after six to twelve hours of ischemia, and necrosis of the skin after from twenty-four to forty-eight hours, and that the cooler the limb during ischemia, the longer the survival time. In this connection, it is of surgical importance to note that collateral arteries dilate in common with arteries generally when sympathetic tone is lost, thus sympathetic section encourages the return of blood to the ischemic tissues.

It is also of surgical importance to note that in the treatment of embolism of

main arteries, if operation is deemed advisable, the sooner it is done the better, the chances of success rapidly decrease after twelve hours, and are almost gone after forty-eight hours have elapsed

An excellent short dissertation on intermittent claudication stresses the points that the cause of the pain is an obstacle to the free passage of blood to the affected limb and that this type of pain is the same as that resulting when normal muscles are made to work with the circulation completely arrested. Then follow short chapters on arteriosclerosis, thromboangitis obliterans, vasoconstriction in general, and Raynaud's disease

In discussing vasoconstriction, Lewis spends much of this book in furnishing his evidence that the traditional view, which attributes much of peripheral vascular disease to a vasospastic cause, is based on inadequate proof, and that attention should not be focused upon the nervous system but upon the blood vessels themselves

Unusual types of gangrene are then discussed, then vasodilatation, and finally vascular disorders in diseases of the nervous system

The whole treatise is written in Lewis' customary clear, simple, authoritative fashion. One wonders at times, however, why he takes such pains to belabor the point continuously that local arterial changes such as minute thromboses are the cause of practically all peripheral vascular disease to the exclusion of primary vasospastic factors. Surgical interference with the sympathetic nerve control has given such unquestioned proof of the importance of the spastic nature of so many of these maladies. With long continued spasm, thromboses may occur as secondary to this partial or complete occlusion of the small blood vessels

I R. T

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# RECENT CONCEPTS IN THE TREATMENT OF HEPATIC DISEASE<sup>1</sup>

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During the last dozen years, the treatment of cirrhosis of the liver has undergone a radical change. This change has resulted in some improvement in the life expectancy of patients with cirrhosis, though not as much as one would like to see. This is due undoubtedly in large measure to our limited understanding of the nature of cirrhosis, but it is due also to the fact that patients present themselves for treatment only after the disease is well advanced.

During the nineteenth century, the usual treatment for cirrhosis of the liver included abstinence from the use of what were usually called spirits, and a diet consisting of fruits, leguminous vegetables, cereals, milk and eggs. The patient was cupped and fed laxatives and diuretics. Finally, as a last resort, abdominal paracentesis was performed (1). The prognosis was almost uniformly bad. Early in the twentieth century, experimental evidence began to appear which seemed to indicate that the ingestion of meat and of fat was harmful to patients with liver disease, whereas carbohydrates exerted a beneficial effect. This view was based on the following types of experimental data. The portal vein of dogs was cut off from the liver and its blood diverted to the vena cava, that is, an Eck fistula was formed. Then, when these dogs were fed raw meat they became severely and often fatally ill (2). Opie (3) demonstrated that when rats were injected with phosphorus they usually survived if fed a high carbohydrate diet composed of oats and sugar, whereas they usually died if fed a diet limited to meat, and would occasionally die if fed a diet limited to fat. When the rats were injected with chloroform instead of phosphorus, a fatty diet seemed to be most injurious. Later, Mann and Magath (4) reported that the administration of glucose delayed the

<sup>1</sup> Presented at the Saturday morning Therapeutic Conference, February 7, 1948



death of dogs whose livers had been removed. Again, Bollman and Mann (5) showed that dogs injected with carbon tetrachloride would die of cirrhosis if fed a diet limited to meat, but would survive the experimental period if fed milk, bread and syrup. All these experiments, and many more like them, led to the treatment of both acute and chronic liver disease with diets rich in carbohydrates and low in proteins and fats. Fig 1 demonstrates the prognosis of 296 patients with cirrhosis who had ascites, at four hospitals under supposedly ex-

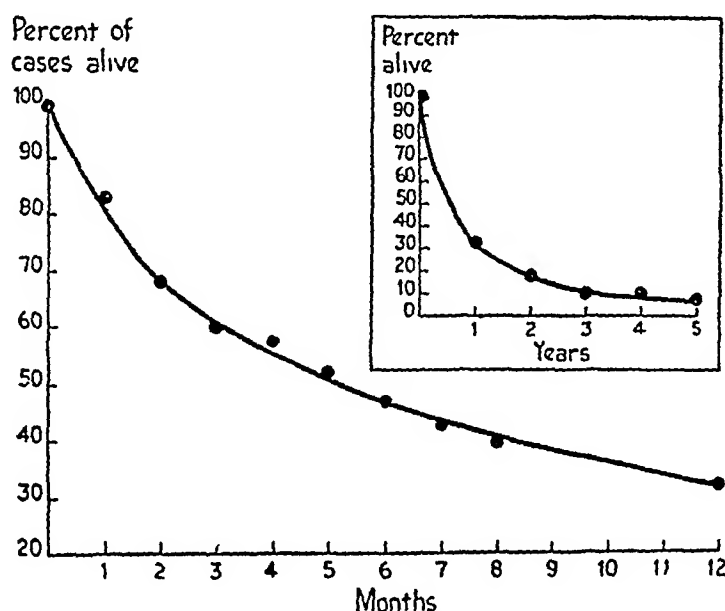


FIG 1 Survival after the onset of ascites in 296 patients with Laennec's cirrhosis treated between 1916 and 1938 (6)

cellent conditions, between 1916 and 1938 (6). Most of these patients were treated with a diet rich in carbohydrate and poor in protein and fat.

In 1937, Patek (7) reported results with a radical departure in therapy. He was impressed by the view, fresh at that time, that the infirmities of alcoholics such as polyneuritis, pellagra, and beriberi were due not to the alcohol consumed but to the concomitant poor dietetic intake of these patients. At the time, there were many enthusiastic reports in the literature concerning dramatic cures of alcoholic polyneuritis with B vitamins. Patek undertook, therefore, to treat patients with

cirrhosis with a diet rich in B vitamins, supplemented by oral and parenteral B vitamins. Ten of thirteen patients fed a balanced diet of approximately 300 grams of carbohydrate, 100 grams of protein, and 120 grams of fat showed striking improvement. This seemed well beyond the chance expectation of improvement in this disease. Patek appreciated the fact that the value of the diet given these patients did not necessarily depend upon any single component, and in further studies an even higher caloric intake of protein, carbohydrate and fat was given. Thus the dietetic regimen of patients under Patek's care now includes approximately 350 grams of carbohydrates, 140 grams of protein, and 175 grams of fat, a total of 3500 calories a day (8). The diet consists chiefly of meat, fish or poultry, milk, eggs, fruit and green vegetables. The diet is supplemented with a total of 50 grams of powdered brewer's yeast a day, or, if this is not tolerated, an oral B complex preparation. In addition, intramuscular injection of thiamine chloride, 5 mgm daily, and unconcentrated liver extract, 5 cc twice weekly, is given. The diets are diversified according to the patient's individual desires, rather than to fit the general hospital routine, since food that is not eaten is of no value to the patient. Furthermore, the patient is spoon fed when necessary by the attendants, nurses and residents, in order to coax the food down the esophagus. Fig 2 shows a recent tabulation of the prognosis of patients with cirrhosis and ascites who were treated with this regime. The survival of these patients is compared with 230 patients of the 1916-1938 series who were alive one month after the onset of ascites. It should be pointed out that the improvement in the prognosis of patients with cirrhosis occurred during the era of chemotherapy for infection, and this may account for some of the improvement noted.

As might be expected, the earlier a patient with cirrhosis is treated and the milder the case, the more readily improvement appears. Indeed, Post and Patek (9) were able to correlate the prognosis of patients treated with a highly nutritious diet with the plasma serum albumin level at the time of the patient's admission to the hospital. On the average, those patients who died had a serum albumin of 2.4 grams per 100 cc on admission, whereas those who improved and whose ascites disappeared had a serum albumin averaging 3.0 grams on admission, a difference which was statistically significant. It cannot be

emphasized too strongly that the treatment of patients with cirrhosis must be continued for a long time, and the fact that improvement does not occur within several weeks should not be cause for discouragement. Fig. 3 demonstrates the length of time from the initiation of treatment until the disappearance of certain specific signs. Patek's observations have been amply confirmed by other observers (10, 11, 12)

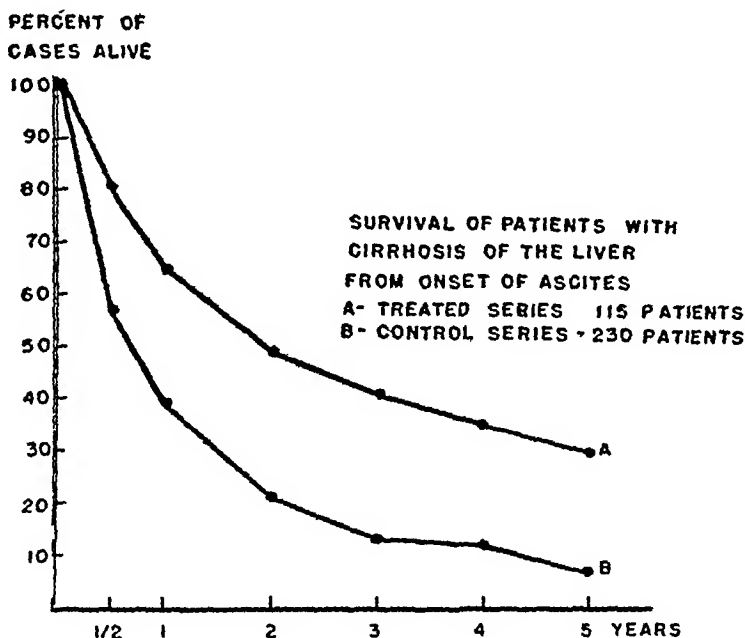


FIG 2 Survival after the onset of ascites in 115 patients with cirrhosis of the liver treated with a highly nutritious regime, compared with 230 patients who lived at least one month after the onset of ascites, and were treated during 1916-1938 (8)

In recent years, experimental evidence has appeared which seems to place the use of a highly nutritious regime on a rational basis. In the first place, Davis and Whipple (13) had shown as early as 1919 that a diet of skim milk or casein protected dogs given chloroform, an observation confirmed in rats by Moise and Smith in 1924 (14). Then, a great flood of reports appeared, by Gyorgy and Goldblatt (15), Rich and Hamilton (16), Chaikoff and Connor (17), Blumberg (18), Spellberg and Keeton (19), Webster (20) and many others, that rats, rabbits, and mice fed special diets developed necrosis of liver cells, fatty in-

filtration of the liver, and an increase in fibrous tissue in the liver. With our current hindsight, we now recognize that these diets were, in general, relatively low in protein and usually relatively high in fat as well. It was quickly found that the lesions could be prevented by increasing the protein content of the diet, or by feeding large amounts

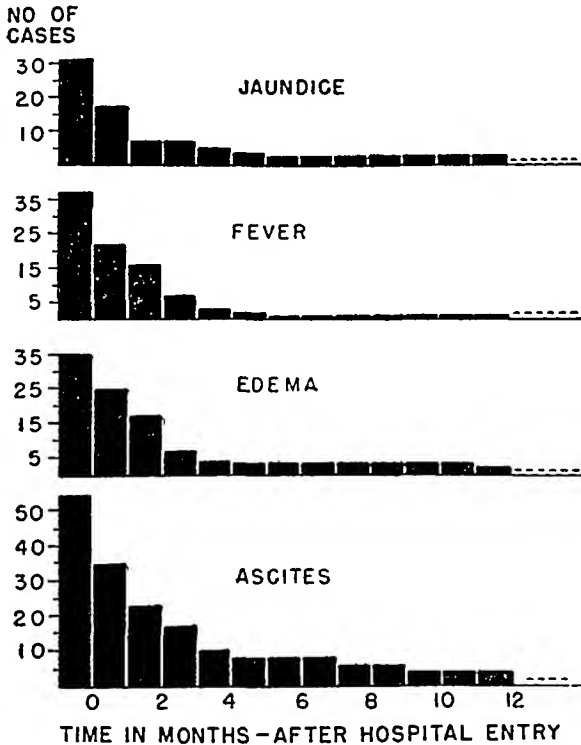


FIG 3 The time at which specific signs of hepatic failure disappeared after the initiation of treatment with a highly nutritious regime (8)

of brewer's yeast (15, 16, 20, 21). Similarly, these substances offered more or less protection against such toxins as arsenic (22, 23) and chloroform (24).

The factors in protein and brewer's yeast which afford protection against dietetic hepatic injury are not yet clearly recognized. One can readily understand this, since the basic diet, the species of animal, and the dose of the adjuvants tested varied from study to study. For example, Daft, Sebrell and Lille (25) reported that choline prevented

cirrhosis in rats fed a low casein, low fat, high carbohydrate diet. On the other hand, Gyorgy and Goldblatt (26) found that choline would not prevent liver injury in rats fed a low casein diet when these rats got most of their calories from lard or Crisco instead of from carbohydrate. However, these rats were protected by a combination of cystine and choline. The protective action of cystine was the more remarkable since previously it had been shown that large amounts of cystine were severely toxic for the liver (27, 28). Methionine was also

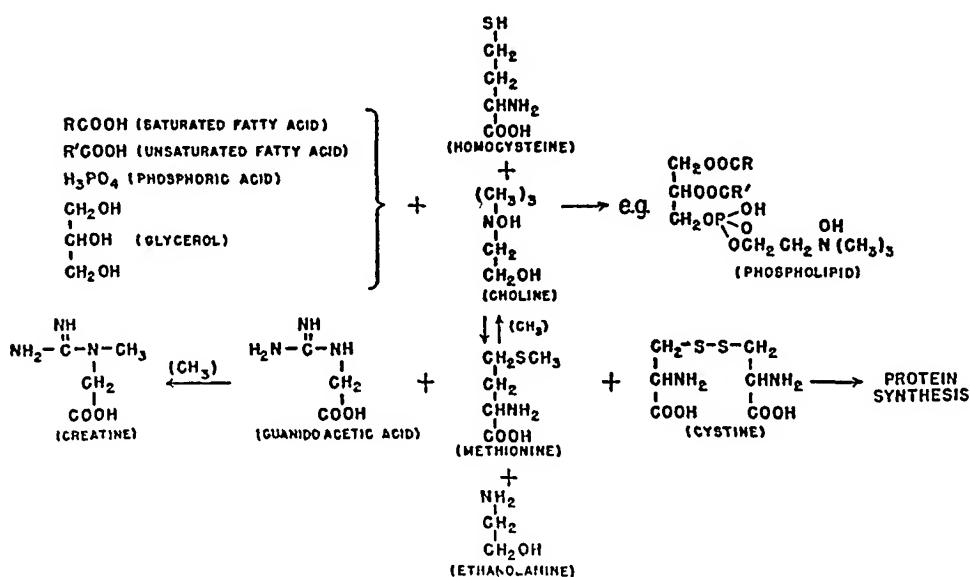


FIG 4 Some inter-relationships between lipid transport and choline-methionine metabolism. Fat may be transported from the liver to peripheral depots as phospholipids. The choline necessary for this, however, may be needed to furnish methionine for creatine synthesis or protein synthesis. In this event, phospholipid cannot be synthesized and neutral fats accumulate in the liver.

shown to protect the liver of rats fed low casein diets (29, 30, 31), a protection potentiated by small amounts of cystine (30). It is of interest that some of the factors which seem to prevent experimental dietetic cirrhosis are the same as those which Best, Hershey (32) and others (33) found would prevent certain of the experimental fatty livers. Fig 4 is a rough outline demonstrating some of the relationships between choline, methionine, and fat transport. To oversimplify it, fatty infiltration of the liver occurs when the neutral fats

cannot be removed from the liver by such transport systems as the phospholipids. The liver is infiltrated with fat whenever the synthesis of phospholipids is impaired. This occurs when there is an insufficiency of essential fatty acids, when there is an insufficiency of choline and methionine, when excesses of cystine cause the utilization of methionine for the formation of protein, and so forth.

The ability of lipotropic substances to prevent experimental dietetic cirrhosis was not entirely unexpected. As Connor (34) emphasized, fatty infiltration of the liver often precedes the development of cirrhosis both in human beings and experimental animals. However, it was not long before it was found that lipotropic substances did not afford complete protection against hepatic injury. For example, Daft and his associates (30) observed that rats fed a low casein diet developed hepatic necrosis as well as periportal fibrosis. Choline or methionine protected the rats against the fibrotic lesion, but had no effect on the necrotic lesion. These necrotic lesions, however, could be prevented by a combination of methionine and cystine. Himsworth and Glynn (35) also emphasized that two separate types of lesions—necrotic and fibrotic—were encountered in rats fed experimental diets. They observed that rats fed a low protein diet developed massive necrosis of an entire liver lobe. This massive necrosis occurred whether or not the liver was infiltrated with fat. Choline did not prevent the necrosis, while methionine in small amounts was completely protective (36). Now, when rats were fed a diet rich in fat and low in lipotropic substances they developed diffuse hepatic fibrosis. Here again, the pathologic sequence of fatty infiltration of the liver and fibrosis of the liver described by Connor was observed. This diffuse fibrosis could be prevented by choline (35), unlike massive hepatic necrosis. Himsworth and Glynn's experiments are not entirely free from criticism. For example, the rats were fed in groups rather than individually, so that the actual dietetic intake of each rat was not known. They were able to repeat their experiments with rats from two sources housed in two separate laboratories, but this does not entirely rule out the possibility that some indigenous infection of rats was uncovered by the low protein diets. To my knowledge, Himsworth and Glynn's experiments have not been confirmed. However, it is probably true that microscopic (rather than massive) necrosis of the liver is a lesion separate

in its origin from fibrosis. Recently Green and Brunschwig (37) demonstrated that a large series of compounds would protect rats against one or the other of the two lesions, but not necessarily both. In summary, then, hepatic lesions can be produced in experimental animals by feeding various diets, and these lesions can often be prevented by special dietetic factors. But neither the pathogenesis, prevention nor treatment of these lesions has really been clarified.

The evidence of the experimental value of choline, methionine, and combinations of choline and methionine with cystine has led to the wide use of these substances as adjuvants to a highly nutritious diet in the treatment of cirrhosis. Suffice it to say, their value under these conditions is unproved. Perhaps this is because any diversified nutritious diet is rich in the foods necessary for the nutrition of the liver. For instance, casein and egg white are said to be rich in methionine (31), wheat gliadin (38), peanut meal, muscle meats and lactalbumin in cystine (31), and meat, beef liver and pancreas, cereal, wheat flour, polished rice and rice flour, skim milk, brewer's and baker's yeast, peanut meal, soy meal and vegetables in choline (39, 40). Since these substances are present in a well balanced diet in amounts which are apparently adequate, it would seem unreasonable to expect much benefit from giving still more to the patient. It is, however, probably fair to say that choline and methionine in moderate doses are not harmful, unlike cystine which is potentially dangerous.

It should be emphasized that experimental diets deficient in these protective substances are achieved only with the greatest of care. Moreover, comparatively slight shifts in the diets of these experimental animals will cause major changes in the incidence of hepatic lesions. For example, Earle and Victor (41) showed that rats fed a diet rich in butter were more susceptible to cystine than rats fed a balanced diet, while a diet rich in lard and cod liver oil was actually slightly protective. In another situation, on the other hand, Gyorgy and Goldblatt (26) observed that choline prevented hepatic necrosis in rats fed butter, but not in rats fed lard or Crisco. In another instance of the importance of the details of experimental diets, Webster (20) observed that small amounts of molasses prevented dietetic cirrhosis in rats. Molasses is a crude carbohydrate rich in inorganic sulfur and containing about 2.5 to 3 per cent protein. In the clinic, the diet is always diversified, and

the addition of one component or another does not seem to matter. Not to labor the point, but the experiments which demonstrated the toxicity of raw meat illustrate the complexity of the experimental problem very well. It was pointed out above that diets limited exclusively to lean meat aggravated the hepatic lesions produced by phosphorus (3) or carbon tetrachloride (5). Bollman and Mann (5) observed that it was not the protein of the meat which was toxic, but rather something in a water extract of the meat. More recently, Ralli and Rubin (42) reported that feeding raw lean beef to depancreatized dogs caused fatty livers, and again noted that the toxic substance was in a water extract of the meat. They discovered this extract contained inositol. When inositol was fed along with meat from which the toxic water-soluble substances had been removed, the dogs developed fatty livers. The presence of inositol, then, seems to account for some of the toxic effects of all-meat regimes. It has been shown that biotin and cholesterol produce fatty livers in rats. Just to complicate the picture further, inositol actually prevents these fatty livers produced by biotin (43) or cholesterol (44). Of course, no one advocates feeding patients a diet limited to lean meat. In the same way, arguments for the use of low fat diets are based upon experiments in which the composition of the diet is completely artificial. In practice, if the amount of fat in the diet is limited, the diet is usually so unpalatable that the patient fails to eat enough of anything.

Recently the use of intravenous liver extracts has been recommended for patients with Laennec's cirrhosis (45, 46). Liver extracts were advocated for the treatment of liver disease as early as 1626 (47). Numerous enthusiastic reports of their use appeared in the French literature early in the present century (49, 50). Recent experience from the Rockefeller Institute and elsewhere with the use of intravenous liver extract has been most encouraging. However, this study may be taken as an instance of the difficulty of interpretation of therapeutic results. In measuring the value of intravenous liver therapy, Labby and his associates (45) eliminated from consideration those patients who did not have at least a six months' course of therapy. Of those patients who had this minimal course, 77 per cent survived for two years, compared with 45 per cent who survived when treated with Patek's regime at that time (51). Now, if the data are studied, two



things become apparent. In the first place, Labby and his associates included all patients with cirrhosis in their statistics, while Patek included only those who had ascites—that is, patients in whom the disease was more advanced. In the second place, Labby and his associates eliminated from consideration those patients who did not survive six months. In other words, Labby's and Patek's data are not comparable. None of this is intended as criticism of one or the other group, but merely to emphasize the difficulties involved in making such a comparison. The use of intravenous liver extract certainly warrants further study. Patek (8) recently treated 6 patients with intravenous liver extract. These patients were in a stable state of decompensation, not responding to at least six months of the basal dietetic regime. The course of three of the patients seemed unaffected by the liver extract. The other three, who had had 33, 75, and 126 abdominal taps respectively, had an increase in appetite and their ascites gradually disappeared. Of course, this is not proof of the efficacy of liver extract. The only way that the value of specific dietetic adjuvants could be tested adequately would be to set up rigidly controlled, long term studies of such enormous proportions as to be economically unfeasible.

Thus far only the dietetic care of the patient with cirrhosis of the liver has been discussed. I should like to touch briefly on the treatment of certain specific symptoms. The factors which result in the formation of ascites are only poorly understood. For some time it was thought that the increase in portal pressure (52, 53, 54, 55) and decrease in serum albumin (56, 57, 58) and colloid osmotic pressure (59, 60) were enough to account for the presence of ascites. Ralli and her associates (61), however, pointed out that diuresis seemed to occur in some patients with cirrhosis before any rise in serum albumin or colloid osmotic pressure. They demonstrated that the urine of cirrhosis patients with ascites contained a large amount of an antidiuretic substance and suggested that this antidiuretic may contribute to ascites formation. However, this work has not been adequately confirmed. Recent work of Patek and his associates (62) suggests that differences in permeability of the portal vascular bed may determine whether or not ascites accumulates. In the treatment of ascites, an attempt is made to raise the colloid osmotic pressure of the plasma by feeding a

diet rich in protein This is a difficult process, however Myers and Keefer (63) and Post and Patek (64) studied the nitrogen balance of patients with cirrhosis Although the patients retained large amounts of ingested nitrogen, the serum albumin level did not rise concomitantly Diuretics have been used in the treatment of ascites for more than a century and are still important weapons At present, mercurial diuretics and ammonium chloride are most frequently employed In recent years, the effect of intravenous injection of concentrated human albumin has received extensive trial If approximately 50 grams a day are given to patients with cirrhosis and ascites, the serum albumin level rapidly rises to normal levels (62, 65, 66), but diuresis is unimpressive Apparently, this is because the albumin diffuses into the ascitic fluid almost as rapidly as it is injected If larger doses of albumin are given, for example, injections of 100 grams at a time, a satisfactory diuresis is said to occur (67) Perhaps this is because the increase in plasma volume which results acts as a diuretic (60) Obviously this increase in plasma volume might be dangerous for patients with esophageal varices Paracentesis, once reserved for the moribund, should be resorted to relatively early in the treatment of ascites, since the ascites interferes with the patient's ability to eat It is well to remember, however, that occasionally a patient will develop cholemia after paracentesis Although many surgical procedures have been devised for the relief of ascites, none has proved uniformly satisfactory

Bleeding into the gastro-intestinal tract must, of course, be treated as a grave medical emergency The patient should be put at rest, but it is wise to be cautious in the use of sedatives, since patients with impaired liver function may occasionally be more sensitive than the average person to opiates and barbiturates Food should be withheld until signs of active bleeding have disappeared Patients should be transfused early with whole blood, before the blood pressure drops and shock becomes irreversible Numerous operative procedures have been suggested for the relief of portal hypertension in patients with recurrent hematemesis For example, Blakemore (55) anastomoses the portal and systemic venous circulations—that is, he creates an Eck fistula Eleven of nineteen patients with portal hypertension who survived the establishment of a portocaval anastomosis had no gastro-intestinal bleeding during the subsequent six months The mortality rate of the

operation is high, being nineteen per cent in the latest analysis (68), and it is particularly high in patients whose portal hypertension is due to cirrhosis. There is, moreover, no evidence that hepatic function is improved by the operation. It is, therefore, doubtful whether this procedure will prove of value in cirrhosis except in the occasional patient whose major difficulty is recurrent hematemesis.

Cholemia, or severe hepatic decompensation, is difficult to treat. Here, perhaps, the use of glucose and vitamins parenterally is justified, for Snell and Butt (69) were able to revive six patients in hepatic coma by this means.

Hoagland and his associates (70, 71) and others (72, 73) have demonstrated that therapy has little or no effect on the course of acute infectious hepatitis. Although the classic American diet for this disease is rich in carbohydrate and poor in fat and protein, they demonstrated that a high fat, high protein regime was not only as effective, but may indeed have shortened the course of acute infectious hepatitis. The use of such adjuvants as crude liver extract and methionine was without benefit. Rest has been advocated (71, 74), and with this we agree, but the evidence which has been presented to substantiate this point of view is probably incomplete.

The question often arises whether the patient in coma from acute infectious hepatitis or cirrhosis should be given intravenous amino acids. On theoretical grounds, this might be considered dangerous since the sick liver might not be able to metabolize the excess load of amino acids. Actually, however, no deleterious effects have been observed from the intravenous use of commercially prepared amino acids in patients in hepatic coma (75) or ill with severe hepatitis or cirrhosis (77, 78), although at the same time one would hesitate to ascribe any benefit to them.

It is probably obvious that any mention of the avoidance of alcohol by patients with liver disease has been neglected until now. This is because there is no evidence that the moderate use of alcohol is deleterious if the patient at the same time eats an adequate diet (51). However, in the patient with cirrhosis, moderation is a rare virtue, and patients should be urged to become teetotallers.

Finally, it is interesting to learn that we have now, so to speak, completed the cycle. After the long period of experimenting with this or

that dietetic restriction, we have returned to the point where Murchison (79) was in 1868. The diet, he said, should include milk, eggs, farinaceous substances, plainly cooked fish and meat, that is, in the light of modern chemical analysis, a well balanced assortment of high protein foods.

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# HEREDITARY MYOCLONUS EPILEPSY

## TWO CASES WITH PATHOLOGICAL FINDINGS

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The literature contains only a small number of pathological reports dealing with hereditary myoclonus epilepsy. The findings lack uniformity and the pathological basis for the condition needs clarification. This report, concerning a sister and brother, presents some interesting features.

*Case I* M T<sup>1</sup> (Ped A-51076, Path 20738) is a 7 year old white female admitted to the Harriet Lane Home on March 21, 1947, in status epilepticus.

The *family history* is of interest in that a maternal uncle died in the third decade in status epilepticus, having had grand mal seizures throughout his life. A brother G T constitutes the second case of this report. Three older half siblings, ages 16, 14 and 11 years are well and have never had any type of seizures.

The *past history* reveals that the child was born after a 16 hours labour requiring version and extraction. Development was rather slow. She sat at 7 months, walked at 2 years and talked at 2½ to 3 years. She has always been dull and slow. A clumsiness in walking was attributed to knock knees and pigeon feet. Measles and shingles were contracted several years before the present illness.

The *present illness* dates from April, 1946 when the child began to have head nodding attacks associated with uncontrollable jerky movements of the extremities. Episodes of falling and dropping things from her hand began 2 months later, these episodes being associated with a rolling upward of her eyes and momentary unconsciousness. On examination in the Harriet Lane Home in October, 1946 she appeared dull. An intention tremor and an unstable gait were recorded. Increased deep reflexes and a doubtful positive Babinski were noted on the right side. An electroencephalogram was interpreted as showing a petit mal and psychomotor pattern without localization. The child was improved temporarily with tridione and phenobarbital therapy. On March 21, 1947 she collapsed while at school, lost consciousness and began to have left sided convulsions which continued until admission 8 hours later.

*Physical examination* revealed the child to be comatose and actively convulsing. There were regular clonic contractions of the left arm and leg, increased deep

<sup>1</sup> The clinical aspects of these two cases will form part of a more extensive report to be published by Dr L Kajdi and Dr S Livingston.

tendon reflexes on the left, deviation of the head to the right and bilateral ankle clonus

Pertinent *laboratory data* included a spinal fluid under increased pressure showing 15 mononuclear cells, protein of 53 mgs per cent, a negative Wassermann and Mastic curve. The blood calcium was normal. There was no evidence by x-ray or blood analysis of lead intoxication. Ventricular air injection revealed no significant abnormality. Electroencephalogram showed a more abnormal rhythm than previously.

The convulsions were controlled with ether and paraldehyde and later the child regained consciousness, but rhythmic myoclonic jerks persisted. The jerks were first on the left side associated with a paralysis of this side which cleared slowly over a period of a month. Then the right side manifested myoclonic jerks and during the stay in hospital all the skeletal muscles were involved at some time as well as both domes of the diaphragm. These movements ceased during sleep. Five days after admission a right craniotomy was performed and revealed distended veins over the leg area and some cortical atrophy. The wound was closed without further interference. The child improved during the first month to a point where she could walk, but was not well oriented. This was followed by another episode of grand mal seizures after which she remained unconscious until her death on August 14, 1947—146 days after admission. Myoclonic jerks continued throughout this interval and there was a terminal episode of pneumonia. Numerous medications were utilized, none of which gave outstanding benefit.

*Autopsy* It was performed 8 hours after death. The body weighs 20 kgs and is 117 cms long. The development and nutrition are normal. Aside from many small yellow foci of pneumonic consolidation in the lungs and a small mucosal nodule in the rectum, macroscopic abnormalities are confined to the head and brain.

The skull and scalp show evidence of a previous left craniotomy with satisfactory healing. The brain was injected with 4.5% formalin through the carotid arteries before removal and was hardened in 10% formalin for 40 days before sectioning. The leptomeninges are slightly thickened over the frontal and parietal lobes. The vessels of the circle of Willis are smooth and patent. There is no gross asymmetry of the brain and no gross abnormality of its fissural pattern. There is slight convolutional atrophy, more marked over the left fronto-parietal area than elsewhere. A coronal section just anterior to the foramina of Monro shows good differentiation of gray and white matter. The white matter is of normal firm texture and the fibre systems, such as the anterior limbs of the internal capsules stand out clearly. The gray matter of the basal ganglia of both hemispheres is of normal appearance and texture. The cortical gray matter of the right cerebral hemisphere averages  $2\frac{1}{2}$  mm in thickness at the crown of each gyrus. On the left, from the level of the superior frontal convolution to the outer limits of the cortex of the island of Reil, the cortical gray matter is thin, measuring  $1\frac{1}{2}$  mm at the crown of the gyrus, and it seems to be separated from the underlying white matter by a spongy cleavage line. The ventricular system is not significantly displaced. The left ventricle



is slightly larger than the right. The ventricular linings are smooth throughout and the choroidal plexuses appear quite normal. From this plane forward to the frontal poles the left sided cortical thinning and partial detachment gradually disappears, but some signs of it can be found at almost the extreme tip of the frontal lobe. There is no other gross finding of note in the frontal lobes.

A coronal section through the anterior portion of the mammillary bodies again shows good differentiation of gray and white matter, slight enlargement of the left lateral ventricle, no enlargement of the third ventricle, and on the left side extensive cortical changes of the type described above, extending from the supero-mesial margin of the hemisphere downward to and including the left superior temporal convolution. The uncus, pyriform areas and fornices appear normal. There are two minimal areas of cortical softening on the right, but nothing to approach the extensive change on the left side. As one proceeds caudally this same phenomenon of marked cortical involvement on the left and minimal patchy involvement on the right persists to the very tips of the occipital lobes (Fig 1). The striate cortex on both sides is well preserved and a clear cut line of Gennari can be seen throughout its extent. There is, in addition, an area of softening which involves almost the entire left thalamus, apparently sparing the anterior dorsal thalamic nucleus and extending downward until it reaches the region of the corpus subthalamicum and the cerebral peduncle. Across this zone of encephalomalacia the mammo-thalamic bundle stands out sharply and is well preserved. The lentiform nuclei of both sides are grossly normal, as are the external and internal capsules and the optic tracts.

There is moderate, but definite asymmetry in the cerebellum, the right cerebellar hemisphere being throughout smaller than the left. The markings of the cerebellar folia of the right hemisphere are accentuated, and the folia themselves appear thinner than do those of the left. The central cerebellar nuclei are possibly less clearly differentiated from the central white matter than is usual, but no gross softening is palpable. The brain stem, floor of the fourth ventricle and upper cervical cord are grossly normal.

*Microscopic findings* The lungs show lobular pneumonia. The liver shows fresh central necrosis and central fatty infiltration. A small benign argenaffinoma is found in the rectum. The retina and choroid of the eyes are not obtained. The remaining microscopic abnormalities are confined to the brain.

The leptomeninges are normal. The sections of cerebral cortex show foci of destruction of gray matter apparently sparing other areas completely. These lesions are more extensive on the left, and are seen in sections from all lobes on this side.

The most extreme lesion is one of such marked encephalomalacia of the cortical gray matter that even the glial framework is rudimentary except in the subpial layer (Fig 2). There is astrocytic hyperplasia of the molecular layer, and a very imperfect meshwork of astrocytes persists in the deep layers. Compound granular corpuscles are abundant. No ganglion cells remain in such an area. The underlying white matter shows great loss of nerve fibers in a Bodian stain, and a marked

astrocytic hyperplasia Nissl's plump astrocytes are abundant, and giant astrocytes with multiple nuclei (Fig 3) are seen These elements are most concentrated at the junction between gray and white matter Fat stains reveal an abundance of fat droplets both free in the tissues and within macrophages

Other areas show less extreme lesions, usually sparing some layers more than others The subpial layer is always partially intact At least a glial framework usually persists in layer II and the upper part of layer III The ganglion cell loss is sometimes laminar in distribution, but is not consistently selective to certain layers Frequently a few ganglion cells persist although the destructive process about them is marked Some areas show considerable loss of neurons and marked alterations of those remaining, but the glial framework is intact and perhaps more dense than normal (Fig 4) Such areas show some capillary proliferation At the junction of gray and white matter a noticeable concentration of Nissl's plump astrocytes is also noted in these less severe lesions Fat laden phagocytic cells are abundant in both gray and white matter An early cortical change is the presence of fat laden cells in areas where the ganglion cells are normal in number and distribution Only in some minimally affected areas are the fat laden cells obviously perivascular All grades of ganglion cell alterations are seen up to complete cytolysis In Nissl preparations some ganglion cells in and adjacent to the lesions show pale staining qualities, chromatolysis, cytoplasmic vacuoles, shrinkage and pyknosis of the nucleus, displacement of the nucleus and karyolysis Some of these ganglion cell changes probably represent post mortem alterations Numerous areas of cortex are apparently free from any lesion Both motor cortical areas are partially destroyed The cornua ammonis on both sides shows occasional laminar spongy areas adjacent to the ganglion cell layers These areas differ from the above described lesions in that they show no evidence of gliosis or cellular reaction, and may represent artefact

Myelin sheath stains show considerable loss of myelin in the white matter underlying involved areas of cerebral cortex with puddling and globule formation (Fig 5)

The left optic thalamus shows the area of encephalomalacia noted grossly (Fig 6) to be greatly rarified by the presence of innumerable contiguous vacuoles, so that it has become a status spongiosus (Fig 7) Besides glial fibres the walls of this meshwork also contain nerve fibres as seen in reduced silver preparations There is a definite increase in mature astrocytes, but no plump astrocytes are seen The appearance suggests a more quiescent lesion of longer standing However myelin sheath stains show puddling and globule formation Fat stains show rather abundant fat laden phagocytes A few foci of surviving neurons are present here and there Some of them show chromatolysis and shrinkage The medial portion of the right thalamus is spared, but the remainder shows the same lesion as the left The region of the corpus subthalamicum shows essentially the same lesion as the thalamus on both sides

The head of the left caudate nucleus shows a few small circumscribed lesions of the same type as those in the thalamus, but its greater portion is normal The

left putamen also contains a few tiny spongy foci and a small associated area with ganglion cell loss and replacement by mature astrocytes. The pallidus on the left is normal. The section of caudate and lentiform nucleus on the right is normal.

There are circumscribed vacuolated areas in the substantia nigra on both sides, similar to those in the thalamus. The ganglion cells remain around these areas. There are lipid laden phagocytic cells in this region, and dorsally in the decussation of the brachium conjunctivum. The pontine nuclei show very small areas of involvement by this same spongy lesion.

The cerebellar cortex is severely damaged. The more posterior portions of the hemispheres are relatively normal, but moving anteriorly toward the cerebello-pontine angle, there is marked destruction and atrophy on both sides. The molecular layer becomes narrowed, the Purkinje cells disappear completely and there is marked cell loss in the granular layer of this portion. There is proliferation of the Bergmann layer. Elsewhere many of the remaining Purkinje cells show shrinkage, a coarsely granular cytoplasm, pyknosis or karyolysis. Fat stains show numerous lipid filled macrophages in the affected gray matter, but very few in the white matter. Some of these are rod cells. Myelin sheath stains, however, show areas of puddling and globule formation in the white matter.

The dentate nucleus also shows a severe lesion (Fig 8). The ganglion cells are somewhat decreased and there is a definite increase in mature astrocytes. The outstanding point is the presence of very numerous large vacuoles. The latter are chiefly localized at the junction between gray and white matter on both external and internal aspects of the nucleus. Many of the remaining ganglion cells show shrinkage, and some show pyknosis, displacement of the nucleus or karyolysis. Some of the cells show partial cytolysis. A fat stain reveals almost no fat laden cells in the region of the nucleus. However, a myelin sheath preparation shows areas of puddling and globule formation within and about the arc formed by the dentate nucleus.

Finally a circumscribed oval lesion 5 mm by 2 mm in diameter is present in the medullary substance of the cerebellum on the left side, about 4 mm lateral to the dentate nucleus. This area is spongy and vacuolated with evidence of gliosis, and resembles the lesions described in the thalamus.

The medulla including the inferior olives is normal. The upper end of the cervical spinal cord is normal, without evidence of degeneration in the projection fibres in myelin sheath stains.

A careful search fails to reveal intracellular or extracellular inclusion bodies. Special stains for iron, including the pallidus, give a negative result. The reliability of glycogen stains is impaired by the form of fixation. Using the periodic acid routine (1), controlled by the saliva test (2), no glycogen can be demonstrated.

*Case II* G T (Ped A-24278, Path 19848) is an 8 year old white, male admitted to the Harriet Lane Home on Dec 22, 1945 in status epilepticus.

The *family history* is described in the case report of sister M T above.

The *past history* revealed a difficult delivery requiring version and extraction,

and artificial respiration was administered for 30 minutes. Development was slow. He sat at 7 months, walked at 2 years but was clumsy. He made sentences at 3 years. The child had whooping cough at one year of age, chicken pox and measles during early childhood.

The *present illness* probably began at the age of 2½ years (20 months after the episode of whooping cough) with occasional falling spells. This was followed soon by attacks of head nodding and rhythmic movements of the arms. There were no myoclonic jerks at this time. The child was seen in the Harriet Lane Home at 4 years of age with vomiting episodes unrelated to meals or obvious psychic causes, but followed by about 2 hours of sleep. A torticollis was noted, but no other abnormalities. The vomiting improved temporarily and he was next seen at the age of 8 years. On Dec. 19, 1945, 3 days before admission there was a recurrence of vomiting and episodes of momentary unconsciousness, the eyes deviating to the right. Twelve hours later clonic jerks, chiefly right sided, began. The child became unresponsive and the jerks continued until admission.

*Examination* revealed the child to be comatose with occasional right sided twitches. The reflexes were depressed. After regaining consciousness examination revealed right sided weakness, intention tremor, poorly performed coordination tests and some mental retardation.

Pertinent *laboratory data* included a spinal fluid which was under increased pressure, but negative on examination. Ventricular air injection was interpreted as negative. Electroencephalogram suggested a spike and wave pattern, the waves being irregular and the rhythm abnormally slow.

The convulsions were partially controlled with phenobarbital and paraldehyde, and consciousness was regained over a 3 day period. The tremor and jerking movements of the right arm persisted but the right sided weakness improved. Generalized convulsions recurred on January 28, followed by an exacerbation of the myoclonic movements in both arms and 3 days of unconsciousness. Following this the pupils failed to react to light, the child was very dull and appeared to be blind. Death on March 19, 1946, followed a febrile episode. This was 87 days after admission. Numerous medications were without significant benefit.

*Autopsy*. These findings are taken from the autopsy protocol and were not witnessed by the present writer. The autopsy was performed on the day of death.

The body weighs 15½ lbs and is 120 cms long. The nutrition is poor. No macroscopic lesions are seen in the viscera.

The brain is fixed in the same manner as that used for the first case. The pia is delicate and can easily be removed from the brain substance. The vessels at the base and the dural sinuses are normal. The cranial nerves appear normal. The convolutions are flattened, but there is no pressure cone. On section the cut surfaces show normal structure grossly and the grey and white substance are sharply demarcated. The lentiform nucleus contains a few more whitish fibres than usual.

*Microscopic Findings*. The lungs contain minute foci of lobular pneumonia.

The liver shows fresh necrosis and fatty infiltration about the central veins. The kidney pelvis and bladder show slight chronic inflammation. The optic nerve, retina and choroid of both eyes are normal. The remaining microscopic abnormalities are confined to the brain.

The material available for review of the central nervous system is limited to sections made from 11 paraffin blocks. Seven sections contain cerebral cortex, 3 of which show hippocampal gyrus and one of which shows the anterior end of the calcarine fissure. One section is through the putamen and pallidus. Another section shows the floor of the third ventricle just anterior to the mammillary bodies, some paraventricular nuclei and the anterior tip of the thalamus. Two sections include cerebellum and dentate nucleus. Half the medulla, including the inferior olive is on one block and there is a block of cervical cord.

Most of the sections of cerebral cortex are normal except for changes in some of the ganglion cells. These consist of chromatolysis, slight swelling, vacuole formation and pyknosis of nuclei. One section of hippocampus shows an area of moderate ganglion cell loss in Sommer's sector of the cornua ammonis. Many of the remaining cells about this area show poor staining qualities, chromatolysis, shrinkage or loss of nuclear outline. Some cells are represented by formless streaks or shadows. Also in the region of the cornua ammonis in two sections laminar areas of spongy rarefaction are seen adjacent to the ganglion cell layers. These areas show no evidence of gliosis or cellular reaction, and may represent artefact.

The cerebellar cortex shows rather marked loss of Purkinje cells. Many of those remaining show shrinkage, pyknosis, karyolysis, or even partial cytolysis. In some areas the molecular layer appears narrowed. Both sections of dentate nucleus show an appearance somewhat similar to that in the first case of this report. There are numerous vacuoles giving a somewhat spongy appearance to the tissue (Fig 9). This change is concentrated at the junction between gray and white matter on both internal and external aspects of the nucleus. The ganglion cells are present in normal numbers, but some of them are severely altered. Swelling, cytoplasmic vacuoles, displacement of the nucleus, karyolysis and cytolysis is observed. As stated above, many of the ganglion cell changes may represent post mortem alterations. There is some increase in mature astrocytes in the dentate nucleus. There is no evidence of demyelination in Weil stains of the cerebral cortex, cerebellum or spinal cord. There are no other lesions seen. Intracellular or extracellular inclusion bodies are not found. An iron stain including the pallidus is negative. The periodic acid routine fails to show glycogen deposits.

#### DISCUSSION

One is struck by the many similarities in the histories of these two siblings, and perplexed by the dissimilarity of the lesions. However, one must hasten to point out that the material now available for histological study in the case of G. T. is limited, there being no adequate sections of occipital cortex, thalamus, mid brain and pons. However

as opposed to the extensive lesions in M T, only lesions in the dentate nucleus, cerebellar cortex and minimal changes in the gray matter of the cerebral hemispheres are demonstrated in G T.

In searching through the histories to elucidate these differences, much remains obscure. The presence of ataxia and an intention tremor in the case of M T 10 months before death would suggest that the cerebellar lesions were present at that time. Perhaps the hyperreflexia and doubtful Babinski toe sign on the right side would indicate pyramidal tract involvement then as well. G T was not examined at a corresponding interval before death. M T survived for 59 days longer than G T after hospitalization became necessary, but it is questionable whether this interval contributed greatly to the more extensive demonstrable lesions in the former. That G T had more widespread lesion than were discovered is suggested by the report that he was thought to be blind and his pupils did not react to light during the final part of his illness. There was apparently little difference between the two cases in the severity of the generalized convulsions or of the myoclonic jerks.

Rather diverse pathological findings have been reported in myoclonus epilepsy, and the correlation between symptoms and lesions is still not entirely clear. The lesions in M T match in severity and extensiveness the most marked lesions described in the literature (3). It is safe to say that G T is much less severely affected. One does note that the dentate nucleus is involved in both cases, a point emphasized by Hodskins and Yakovlev (4). Intracellular inclusions were not found in either of the above cases, these bodies being frequently noted, but not invariably observed or specific for myoclonus epilepsy (5, 6). Deposits giving the staining reactions of glycogen have been noted in several cases of myoclonus epilepsy (7, 8). The method used failed to demonstrate glycogen in these cases. Iron deposits, especially in the pallidus, substantia nigra, and dentate nucleus are reported (9), but were not seen in these two instances.

From the pathological viewpoint the two cases under consideration might be considered as primary gray matter degenerations if one disregards a lesion in the case of M T in the medullary substance of the cerebellum. The destructive process, however, is more severe in M T than it is in the cases of degeneration of the cerebral gray matter.

reported by Somoza, Alpers (10) and Freedom (11) Examination of the sections of the case described by Ford (12) shows a process in the cortical gray matter very similar to that described for M T The cerebellum was also involved, but the basal ganglia showed no definite alterations in this case Myoclonus was not noted in any of these cases although other hyperkinetic manifestations such as choreiform and athetoid movements were recorded, as well as epileptiform seizures and mental retardation

The similarities between the lesions in M T and the central nervous system lesions reported several days after exposure to severe anoxia (13, 14) are striking enough to bear examination Animal experiments with anoxic anoxia (15) or the anemic anoxia of carbon monoxide poisoning (16) also demonstrate this similarity The destruction of ganglion cells or even complete softening, the demyelination, the prominent microglial response and astrocytic proliferation are features of her lesions which are reported when longer intervals elapse following anoxia The distribution of these changes is also compatible The patchy involvement of the cerebral cortex with a tendency to be laminar, the involvement of the basal ganglia including the thalamus in some instances, as well as the cerebellum and some foci of gray matter in the brain stem fits the distribution of lesions in M T very well The fact that severe hypoglycemia causes lesions like those of anoxia might indicate that such lesions are not specific for one type of metabolic disturbance, although some authors feel that anoxia is the fundamental defect underlying these lesions also (17)

However, it is not apparent that this child suffered from severe anoxia unless one implicates her convulsive seizures Seizures usually leave no recognizable lesions, although lesions are reported in convulsive disorders (18, 19) in the cerebral cortex, especially the cornua ammonis, the cerebellum, caudate, putamen, pallidus, and also in the thalamus (20) They are minimal by comparison with those under consideration

In considering epileptogenous foci, Penfield and Erickson (21) cite cases where a more severe damage was demonstrated and considered to be the result of seizures originating at the involved area They suggest not only that the metabolic demands of the implicated neurons may exceed the temporary circulatory increase during the seizures,

but that these neurons may sometimes die as a result of the process. Other interpretations for these findings remain as possibilities, and in the absence of adequate confirmation one hesitates to take a stand on this evidence.

Both children had histories of a difficult birth, and in G. T. artificial respiration was required for 30 minutes. The evidence that the lesions are recent and progressive is too strong to permit a consideration of birth anoxia or injury as an etiological agent.

Finding ourselves unable to incriminate anoxia as a cause of the lesions, we must for the present regard the lesions as effects of a cause that is not yet recognized, this cause acting in different degrees in different cases. This underlying factor, whatever it may be, possibly consists in some hereditary metabolic disturbance of nerve cells, as is thought to occur in amaurotic family idiocy.

The pathogenesis of the diffuse central necrosis of the liver seen in both cases is not clear. This lesion, which was obviously terminal, is not mentioned in the previously reported cases of myoclonus epilepsy. Fatty infiltration without necrosis is described in the case of Frigerio (22). There is no stage of Wilson's hepato-lenticular degeneration which is known to give this lesion (23). Central necrosis is not a usual finding in deaths associated with the common types of epilepsy (24), but may be seen occasionally without any obvious etiological factor on examining the sections from a group of such cases.

#### SUMMARY

The clinical and pathological findings in two cases of hereditary myoclonus epilepsy are reported. The similarity of the histories of these 2 siblings as opposed to the dissimilarity in the extent of the lesions is pointed out. The lesions of the more severely involved case resemble those reported following severe anoxia, but no conclusive evidence incriminating anoxia can be summoned.

The author wishes to express gratitude to Dr. A. R. Rich, Dr. A. E. Walker and Dr. D. B. Clark for advice and criticism.

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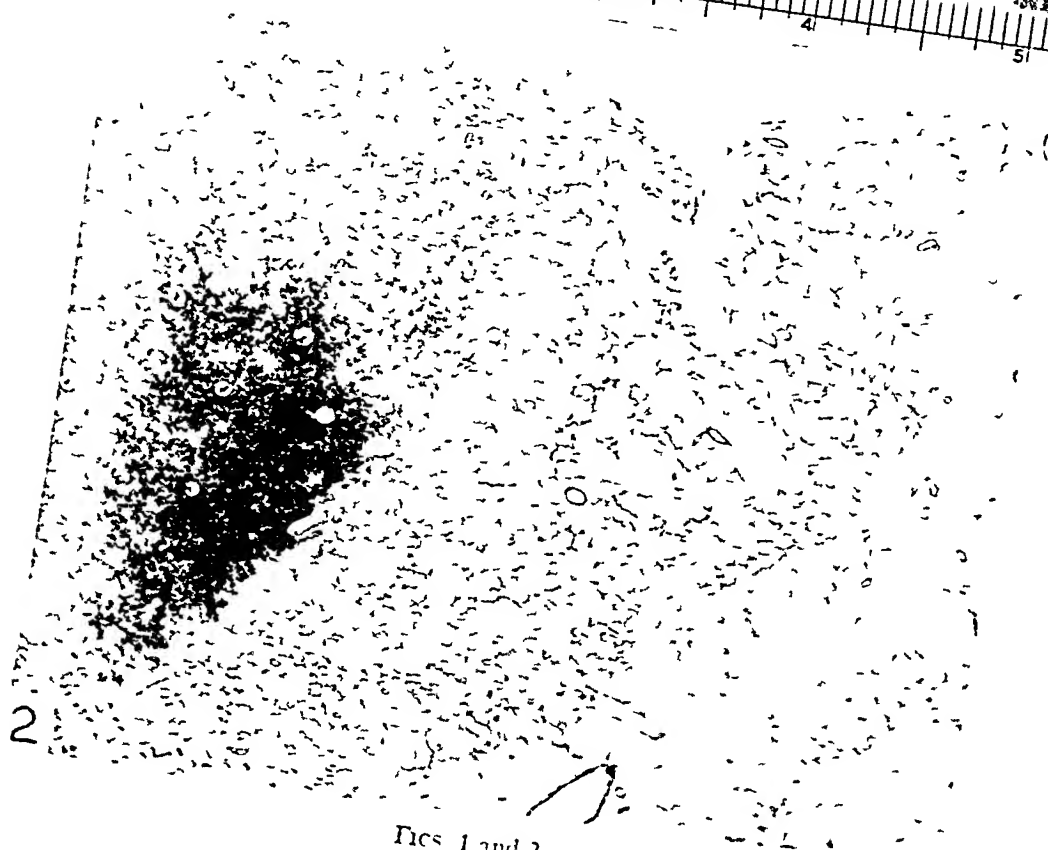
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FIG 1 Case M T Coronal section at the level of the splenium of the corpus callosum showing the thin cortical gray matter on the left side

FIG 2 Case M T Marked encephalomalacia of the cortical gray matter. Astrocytic hyperplasia is most marked in the molecular layer and at the junction of the gray and white matter H and E  $\times 20$

m of the corp.  
gray matter  
at the junction



Figs 1 and 2

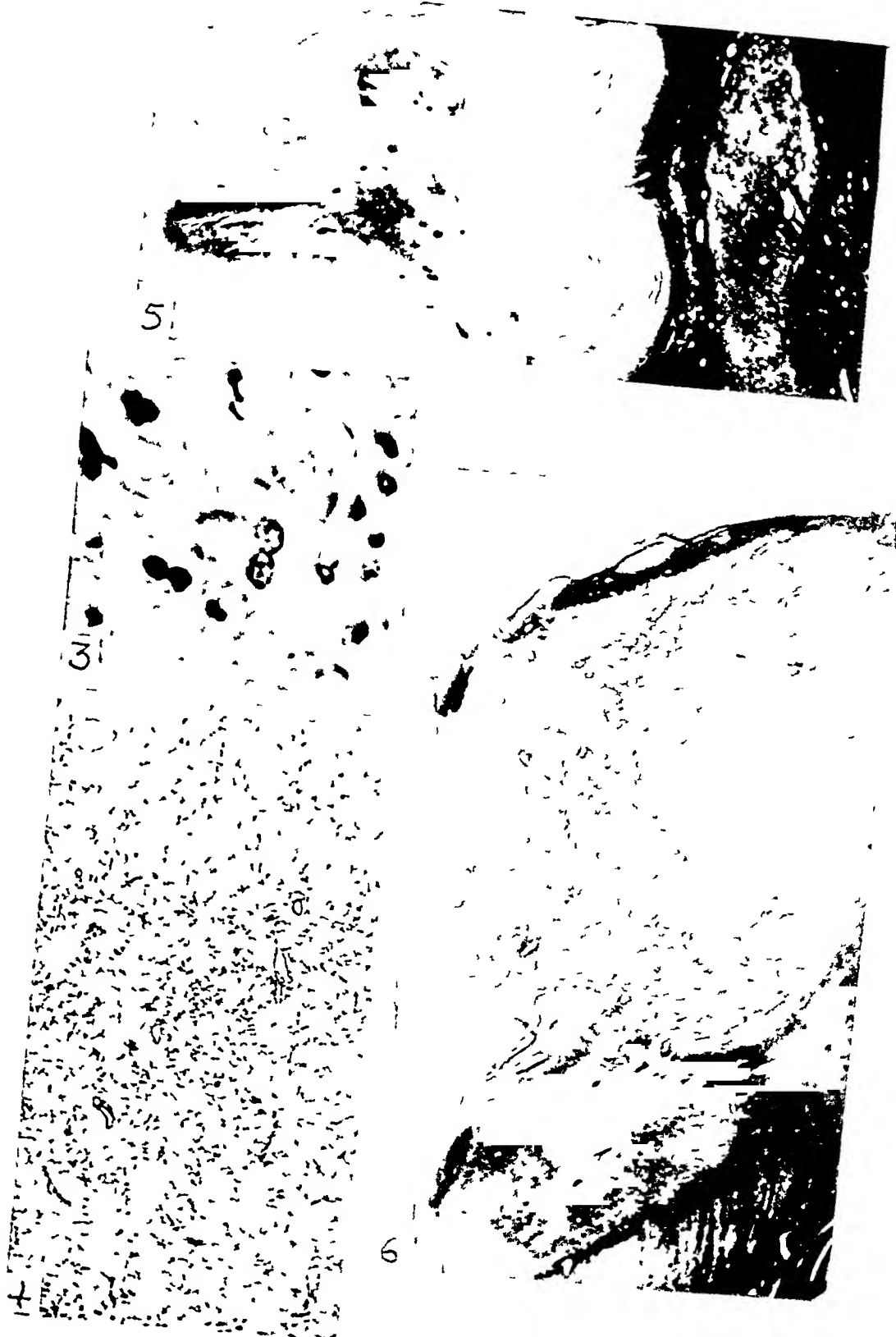
FIG 3 Case M T Large binucleated astrocyte at the junction between the cortical gray and white matter H and E  $\times 500$

FIG 4 Case M T Less extreme lesion showing the more superficial cortical layers The few remaining ganglion cells are altered and the glial elements are more dense than normal Toluidine Blue  $\times 60$

FIG 5 Case M T Loss of myelin in the white matter underlying an involved area of cerebral cortex Weil Myelin Sheath  $\times 3\frac{1}{2}$

FIG 6 Case M T Status spongiosus involving most of the left thalamus Weil Myelin Sheath  $\times 5$

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Figs 3 4 5 6

FIG 7 Case M T Status spongiosus of thalamus A single surviving neuron may be seen in centre field H and E  $\times 100$

FIG 8 Case M T Dentate nucleus, showing numerous large vacuoles chiefly localized at the junction between gray and white matter on both external and internal aspects of the nucleus H and E  $\times 60$

FIG 9 Case G T Dentate nucleus, showing numerous vacuoles similar to those in the dentate lesion of M T H and E  $\times 12$

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# OBSERVATIONS ON THE CLEARANCE METHOD OF DETERMINING RENAL PLASMA FLOW WITH DIODRAST, PARA-AMINOHIPPURIC ACID (PAH) AND PARA - ACETYL - AMINOHIPPURIC ACID (PACA)<sup>1</sup>

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In the course of an attempt to adapt to ambulatory subjects the clearance technique of determining renal plasma flow as originally devised by H. W. Smith and associates, it became evident to us that close scrutiny of the assumptions and methods involved was necessary. Our original purpose was to eliminate the constant infusion of diodrast, or para-aminohippuric acid (PAH), usually used to maintain a stable plasma concentration of the substance used for clearance determination, in order to be able to measure renal plasma flow on ambulatory subjects during different types of exercise. It would be convenient in such circumstances to be able to give a single injection of a substance and determine the renal plasma flow with a falling plasma concentration.

The observations to be presented were made because the clearances of diodrast and PAH in certain circumstances in man under basal conditions were neither constant nor independent of the plasma concentration. Some of the factors which might cause variation in the clearances were studied.

It is possible to determine glomerular filtration rate either with constant plasma concentration maintained by infusion or with falling plasma concentration after single injections of inulin, mannitol<sup>2</sup> or thiosulfate (1, 2). The fall in plasma concentration after single

<sup>1</sup> This work was supported by a grant from the Life Insurance Medical Research Fund.

<sup>2</sup> It is recognized that there has been recent evidence that there may be a small error in the mannitol clearance measurement of glomerular filtration rate.

injections of these substances follows a consistent logarithmic relationship with time, so that the plasma concentration for calculation of clearance can be obtained graphically from the line plotted from a few plasma analyses. With the subject in a basal state, the clearances of inulin, mannitol or thiosulfate are constant and independent of the plasma concentration, whether or not the concentration is held at any constant value or varies continuously throughout the clearance determination.

It is necessary for the validity of the clearance determination of renal plasma flow that the clearance be independent of the plasma concentration. The representation given by H. W. Smith et al. (3) of the relationship between plasma clearance and plasma concentration of a substance used for determination of renal plasma flow is described in Figure 1. Ideally, there exists a range of plasma concentration where the clearance is maximal and constant and is identical with the renal plasma flow. This relationship as represented implies that the clearance is constant and independent of concentration whether the plasma concentration is constant or varying within the maximal range.

"Independence" of plasma concentration can be defined in several ways. First, the clearance may be considered to be independent of plasma concentrations maintained at constant but different levels. This may be demonstrated by stepwise variation of the plasma concentration either upwards or downwards, determining the clearance while the concentration is held temporarily constant (*constant concentration—stepwise variation*). Secondly, the clearance may be termed independent of a *continuously changing plasma concentration*. "Independence" may be qualified in terms of the direction of the change in concentration (*continuously rising or continuously falling concentrations*). Thirdly, the magnitude of the *range of plasma concentration* throughout which the clearance is independent may be different for each substance. This range will be limited by technical as well as physiological factors. At higher plasma concentrations there will be physiological self-depression of the clearance, at very low plasma levels the methods of analysis may be inaccurate.

If the plasma concentration is elevated sufficiently to cause depression of clearance, and then lowered so that clearance rises to its original or maximal value, another type of "independence" is demonstrated.

The *depression* of clearance is then said to be *completely reversible*, and hence the clearance at any given plasma concentration is *independent of previous plasma concentrations*

Failure of the clearance to be "independent" when the plasma concentration is changed by any of the above methods may be due either to a real change in the renal plasma flow, or merely a change in clearance due to factors other than the renal plasma flow. In the first instance, the clearance is still a valid measure of renal plasma flow, in the second instance, the measurement is not valid

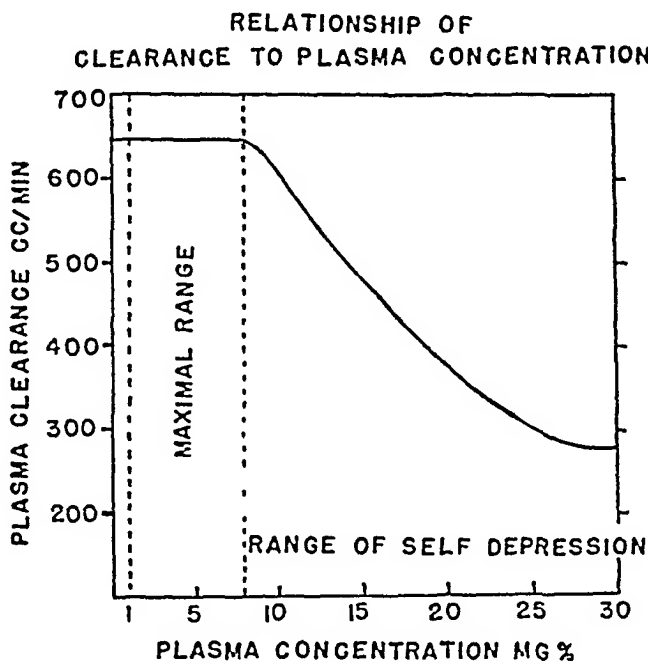


FIG 1

It is conceivable that the substance injected itself may change the renal plasma flow, and that the magnitude and direction of the change depends on the plasma concentration. Either the injected substance or the simultaneously administered solutes could cause variations in body hydration, which may in turn alter the renal plasma flow. In ideal circumstances, the *actual* renal plasma flow should be independent of the plasma concentration of the substance injected, and salt, glucose and water injected with the substance ought to have no effect on renal hemodynamics

There are several hypothetical circumstances which could produce invalid clearance measurements of renal plasma flow. One of the important assumptions involved in the measurement is that there is nearly complete<sup>3</sup> extraction of the substance from the plasma of low concentration flowing through the kidney. If the extraction is not complete, but is a constant fraction of the amount reaching the kidney, the clearance would not be valid quantitatively, but would change in direct proportion to the renal plasma flow. Incomplete extraction or failure of the renal tubules to remove all of the substance flowing through the kidney is demonstrated clearly by self-depression of the clearance at high plasma concentrations when the renal tubules are *overloaded* with the substance. Other causes of incomplete extraction, when the tubules are not overloaded, may be either a *primary failure of the tubular secretory mechanism* or the interference by another substance which inhibits or competes for the secretory mechanism (*secretory blockade*). Such a competing substance may be some of the original compound which has been modified in the body.

Indirect evidence for establishing complete extraction can be obtained by demonstrating a high clearance (i.e. that clearance value which is highest most nearly approximates the renal flow, assuming that falsely high clearances due to manufacture of the substance by the kidney do not occur).

Direct evidence for establishing complete extraction can be obtained by analysis of simultaneous arterial and renal vein plasma. However, demonstration of complete extraction by renal vein plasma analysis is not complete proof that the clearance measures renal plasma flow.

Chemical alteration of the substance might take place in the kidney so that its presence in the urine or in renal vein plasma would not be detected by the usual method of analysis. Complete proof that a substance measures renal plasma flow would require the demonstration of complete extraction, and, in addition, that the extracted substance was collected *completely* and *simultaneously* in the urine in a measurable form.

Here two secondary factors may possibly cause error and invalid

<sup>3</sup> Complete except for a small amount of blood which goes through non-secretory tissue of the kidney.

measurements The first is the possible discrepancy between arterial and venous plasma concentrations With a falling plasma concentration, arterial concentrations would be lower than those obtained from the brachial veins since the tissues of the arm would be contributing their store to the circulation The magnitude of the gradient between arterial and venous concentrations might vary with the rapidity of the fall in arterial concentration and with the rate of diffusion of the substance from tissues to capillary blood Since the kidney would receive the substance from arterial plasma, any discrepancy between arterial and brachial venous plasma concentrations would cause the clearance calculation to be falsely low

The second factor interfering with simultaneity of plasma concentration and the collection of the urine is the *delay time* In all probability the delay time is entirely the time required for the urine leaving the collecting tubules in the kidney to traverse the calyces, renal pelvis and ureters to the bladder With a falling plasma concentration a significant delay time would result in a falsely high clearance calculation because urine which was formed at a higher plasma concentration would be collected simultaneously with plasma at a later lower concentration It is conceivable also that the delay time might change significantly during the experiment due to increase or decrease in urine flow A decreasing urine flow would result in a storage of some substance in the urinary tract and a fall in clearance calculation, conversely, an increasing urine flow would release some of the stored substance and increase the clearance

## METHODS

### (1) *Serum and Urine Determinations*

Mannitol, inulin and PAH<sup>4</sup> were determined in serum or heparinized plasma and urine by the methods suggested by Goldring and Chasis (4), with the exception that precipitation of proteins was carried out by Somogyi zinc sulfate-sodium hydroxide instead of by cadmium sulfate reagents Diodrast was determined by the method of Alpert (5) using this same method of precipitation of plasma proteins

<sup>4</sup> The following abbreviations will be used Diodrast—D, Para-amino-hippuric acid—PAH, Para-acetylaminohippuric acid—PACA, the conjugated product of PAH formed in the body—"PACA"

The determination of PACA or of PAH conjugated in the body ("PACA") required some investigation before complete recovery of PACA was obtained. PACA was determined as PAH after hydrolysis, and the concentration of PACA was calculated from the difference in PAH concentration before and after hydrolysis in the same specimen. Heat and acid required for hydrolysis may cause loss of PAH. The method finally developed is a slight modification of Smith's (9), as follows:

Ten cc of protein-free filtrate or diluted urine containing not more than 0.04 mg nor less than 0.01 mg of PAH are placed in a test tube<sup>5</sup> and 2 cc of 1.2 N hydrochloric acid are added. About 0.5 cc of melted paraffin is dropped on top of the fluid in each of the tubes. The tubes are placed in a boiling water bath for 90 minutes with a rubber stopper loosely inserted. The tubes are then cooled and rotated so that the paraffin sticks to the walls. Addition of the usual amounts of nitrite, sulfamate, and coupler is then carried out in the test tubes for the determination of PAH. It is unwise to add acid to the tubes for the determination of free PAH unless the whole determination is completed immediately, because conjugated PAH in urine may be partially hydrolyzed by standing at room temperature with acid added.

While it is likely that the conjugated PAH found in human serum and urine after the injection of PAH is acetylated PAH, as far as we know it has not been definitely identified and shown to be the only conjugate produced. While the method of determination has been shown to be accurate for PACA, it is assumed to be valid also for PAH conjugated in the body.

Blank determinations for diodrast, PAH and PACA were made on serum and urine obtained before the administration of these substances. No blank substances for diodrast were found in human serum. The blank serum determinations for PAH and PACA varied from 0 to 0.05 mg % and were subtracted from subsequent determinations. Urine blank determinations for all substances were made in order to rule out the possible presence of interfering substances such as sulfonamides. The values for urine blank substances are negligibly small in the final calculations of clearance. The serum blanks for

<sup>5</sup> Thin walled, 16 mm inside diameter, and 220 mm long

mannitol, thiosulfate and inulin were always determined and subtracted from subsequent values

Creatinine was determined on tungstate filtrates of serum and on diluted urine. Serum proteins were precipitated by adding 2 cc of serum to 22 cc of water, then 8 cc of 5% sodium tungstate and 8 cc of  $1/3$  N  $\text{H}_2\text{SO}_4$  solution. To 10 cc of the serum filtrate or of diluted urine, 5 cc of alkaline picrate solution was added and the color read with a photoelectric colorimeter (520  $\mu$  filter). (Alkaline picrate solution, made fresh and used within five minutes after preparation, contains one part of 10% NaOH and five parts of picric acid solution containing 11.75 gm of picric acid per liter.)

(2) *Preparation of Para-acetylaminohippuric Acid for Parenteral Use*

A 20% solution of sodium salt of PAH from ampoules<sup>6</sup> is diluted with an equal volume of pyrogen-free distilled water. Acetic anhydride in slight excess of the ratio of two mols to one of PAH is added while stirring, and the solution is allowed to stand at room temperature for 30 minutes with occasional shaking. The mixture is cooled in an ice bath and filtered by suction through a Buchner funnel. The precipitate is washed several times with ice-cold pyrogen-free distilled water, then with 95% ethyl alcohol, and spread out on dry filter paper to dry in room air overnight. Further drying can be carried out in a desiccator. The precipitate should be protected from light during the drying and stored in a dark bottle.

The substance causing the yellow color sometimes seen in PAH solutions is completely removed by the acetylation since it appears in the washings and the resultant precipitate is white. The yellow color in PAH solutions is produced by some unidentified impurity—possibly an oxidation product.

For intravenous administration, a 5 or 10% solution of PACA was made by dissolving the PACA in NaOH until the pH of the solution was approximately 6.0. (This requires roughly 5 cc of 0.75 N NaOH per gram of PACA.) The solution can be sterilized by filtration or by autoclaving. Solutions of PACA made in this way are free of PAH if filtered, but show traces (0.1%) of PAH if autoclaved.

The solubility of PACA at varying pH's is important because it is

<sup>6</sup> We are greatly indebted to Sharp and Dohme, Philadelphia, for generously supplying ampoules of PAH and mannitol for parenteral use.



conceivable that in acid urine crystalluria might occur. The solubility of PACA was determined. At pH 5.0 in water at room temperature the solubility of PACA was 6.0 grams/100 cc. Within the physiological range of urine pH, precipitation should not occur in urine containing concentrations which are obtained during clearance studies with low plasma concentrations and an adequate urine volume.

### (3) *Clinical Procedure*

Patients were examined in the fasting state in bed. Single injections of substances were given by syringe at the rate of 4 cc per minute. About 20 minutes after the end of the injections, plasma and urine collections were started. Constant infusions were maintained by a gravity drip regulated by a tunnel clamp on the tubing. The rate of drip was regularly and frequently counted and recorded to assure constancy. Urine flow was kept as high and as constant as possible by administration of water by mouth to minimize delay time or storage in the urinary tract.

For calculation of clearances with a falling plasma concentration, the concentration at the mid-time of the urine collection period was used. This was obtained from a graph of the plasma concentrations which yields nearly a straight line on semi-log paper (e.g. Figure 6 and Figure 11). No correction for delay-time was made in any of our calculations. Application of a delay-time correction to any of the falling clearances does not alter significantly the direction or magnitude of the change in clearances. Also no attempt was made to introduce corrections for changes in urine flow since diuresis was maintained.

Metycaine was used as a local anesthetic whenever necessary. Unlike procaine, it does not interfere with PAH determination. Preventing the anticipation of possible pain on repeated vein punctures may be important in maintaining equanimity during the clearance procedures. Urine was collected by catheter in hospital patients and the bladder washed with water and air to insure accurate collections. Normal subjects were allowed to stand to void. Other details of experiments are given with the charts and captions.

In dogs similar techniques were used. Creatinine was administered by mouth two hours before the experiments.

## RESULTS

*Observations on Diodrast*

The first observations in man of a falling clearance occurring with falling plasma concentration were obtained after single injections of diodrast (Figure 2). These experiments raised the questions as to

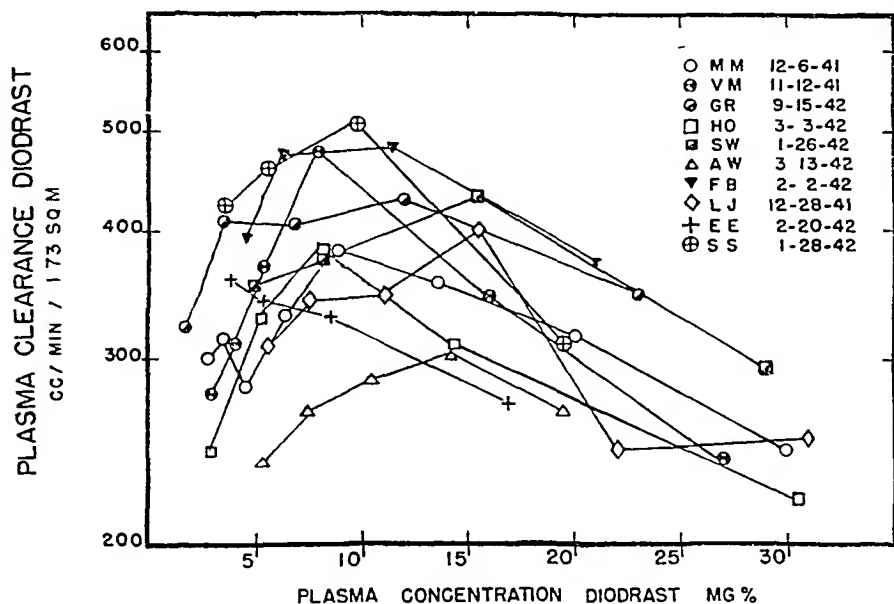


FIG 2 The clearance of diodrast determined with falling plasma levels of diodrast after the single injection of a large amount in ten patients. As the plasma level fell from high levels, where there was depression of clearance, to the region of 10 mg %, the clearance rose. But as the venous plasma level fell below 10 mg % into the range where clearance should be maximum and constant, the clearance fell progressively.

whether the depression of the clearance at low plasma concentrations was caused by the continuous changing of the concentration or whether the depression of clearance was caused by the exposure of the tubules to a previously higher concentration. Accordingly, we attempted to determine what effect exposure of the kidney to higher plasma concentrations would have on the clearance determined with constant plasma concentrations.

In Figure 3 is the result of temporarily raising the diodrast plasma

concentration and then returning to a low sustained level. The clearance was slightly self-depressed when the plasma concentration was raised from 1.0 to 8.0 mg %, but was further depressed when the

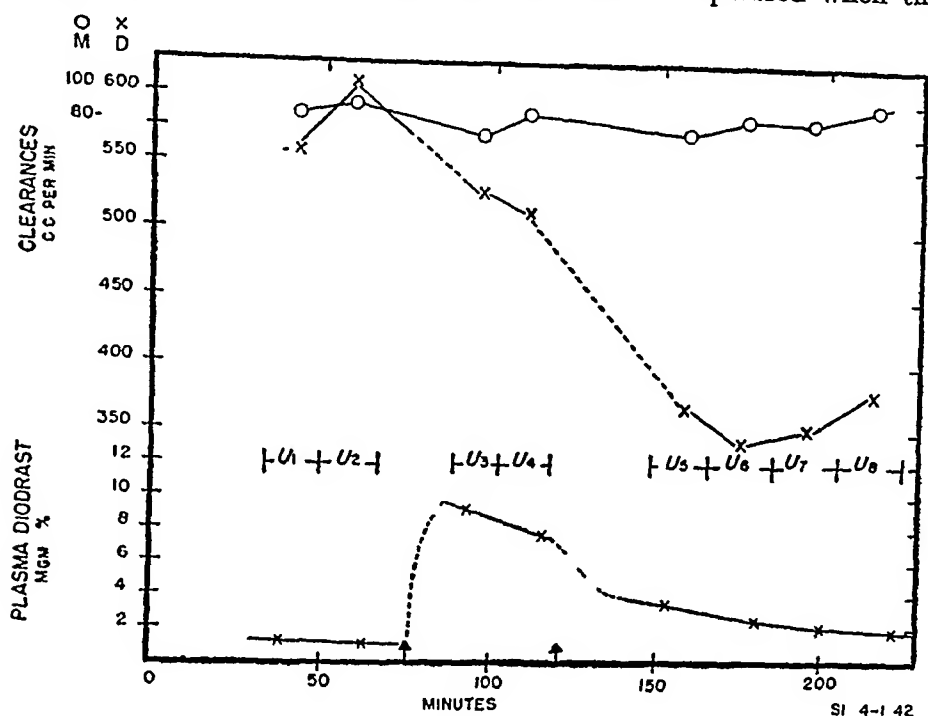


FIG 3 The effect on the diodrast clearance of a normal man of raising and lowering the plasma diodrast concentration by continuous intravenous infusions. Simultaneous mannitol clearances were also determined.

Patient, a 17 year old male, height 71 inches, weight 142 lbs

O = Mannitol clearance

X = Diodrast clearance (above) and plasma concentration (below)

Protocol: Time = 0 minutes—Prime 50 cc of 50% mannitol and 1 cc of 35% diodrast solution, Time = 10 minutes—Sustaining infusion begun containing 3 cc of diodrast and 37 cc of mannitol solution in 150 cc saline at 2 cc per minute, Time = 70 minutes—Prime of 9 cc of diodrast solution given and sustaining infusion changed to infusion containing 27 cc of diodrast and 37 cc of mannitol in 150 cc of saline at 2 cc per minute, Time = 120 minutes—Sustaining infusion changed to solution used originally during time 10 minutes to 70 minutes

plasma concentration returned to a sustained low level. The mannitol clearance remained constant throughout the procedure, indicating that the patient was in a steady state and that the urine collections were accurate.

A similar observation on another patient is recorded in Table I. Elevation of the plasma diodrast concentration to 8.0 mg % caused no self-depression of clearance in this patient. In this case the infusion was stopped abruptly. The clearance determined later while the plasma concentration was falling slowly was markedly depressed.

After these observations, the study was interrupted for a few years and the use of diodrast was abandoned for para-aminohippuric acid, which is determined more easily chemically.

TABLE I

*The Effect of Raising and Lowering the Plasma Concentration on the Clearance of Diodrast in a Patient*

TIME	PLASMA CONCENTRATION	CLEARANCE DIO- DRAST	URINE FLOW	DURATION OF URINE COLLECTION
<i>Mins</i>	<i>mg %</i>	<i>cc /min</i>	<i>cc /min</i>	<i>Mins</i>
22	2.18	515	5.4	19.3
44	1.42	522	10.8	18.3
80	8.3	503	11.2	16.5
98	7.5	554	11.1	21
132	3.4	425	10.5	16.2
150	2.8	290	11.0	16.0

Protocol: At time = 0 minutes—Prime 1 cc of 35% diodrast and infusion begun containing 3 cc of diodrast in 150 cc saline at 2 cc per minute. At time = 55 minutes—Prime 9 cc of diodrast and infusion changed to solution containing 27 cc of diodrast in 150 cc saline at 2 cc per minute. At time = 110 minutes—Infusion stopped. Patient M. M., 12/13/41, height 67 inches, weight 135 lbs.

### *Observations on PAH*

Single injections of PAH in several individuals also produced falling clearances (Figure 4). The plasma concentrations initially were not as high as those obtained with diodrast injections (Figure 2), so that the self-depression of clearance at high concentration is not illustrated. A fall in clearances occurred throughout the range of plasma concentration where the clearance is thought to be maximal and constant.

Lack of reversibility of PAH clearance to original high normal value after temporary elevation of the plasma concentration can also be demonstrated (Table II). The clearance remains low even though

the plasma level is sustained by a constant infusion at a lower level than used previously when the clearance was maximal

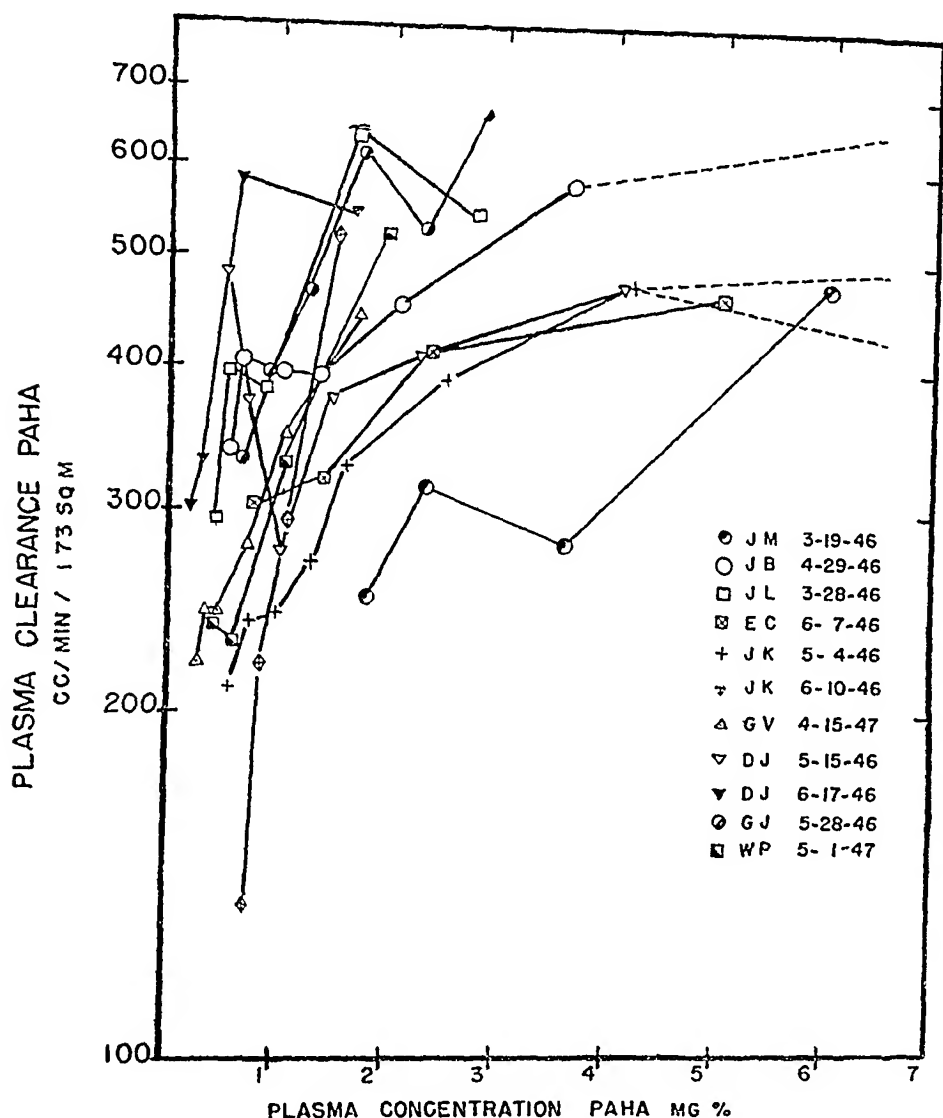


FIG 4 The clearance of PAH falls as the plasma concentration falls after single injections in eleven subjects

The behaviour of the clearance of PAH was observed in dogs given single injections. In the dog, the clearance of PAH does *not* fall when the plasma concentration falls throughout the low range (below 8

mg %), where clearance theoretically should be maximal (Figures 5 and 6) Thus, the PAH clearance behaves differently in man and dog after single injections

TABLE II

*Depression and Lack of Reversibility of PAH Clearance during Constant Infusion Due to Temporary Elevation of Plasma Level*

Normal Male Subject, J M , Age 25, Surface Area 1.9 Sq M

Date 6/5/46

Calculated normal plasma clearance 760 cc/min

TIME	PROCEDURE	PAH PLASMA CONC MID PERIOD	PLASMA CLEARANCE PAH	URINE FLOW
		mg /100 cc	cc /min	cc /min
8 03	I V drip PAH 8 mg /min started			
8 06	2 cc PAH 20% I V prime			
8 16	Urine discard			
8 30	U <sub>1</sub>	1 95	590	4 55
8 45	U <sub>2</sub>	1 52	730	13 8
8 51-8 56	15 cc PAH 20% I V Drip PAH discontinued			
9 07	Urine discard			
9 24	U <sub>3</sub>	12 44	381	15 5
9 44	U <sub>4</sub>	5 1	252	18 5
10 04	U <sub>5</sub>	2 4	460	15 3
10 24	U <sub>6</sub>	1 46	436	6 0
10 27	I V drip PAH 8 mg /min started			
10 40	U <sub>7</sub>	0 97	410	7 9
11 04	U <sub>8</sub>	0 83	464	9 5
11 24	U <sub>9</sub>	0 90	454	12 5
11 44	U <sub>10</sub>	0 96	470	12 3
12 04	U <sub>11</sub>	0 97	496	9 2

The effect of temporary elevation of the plasma concentration of PAH upon the clearance determined at low sustained concentration was not investigated in the dog

The constancy of PAH clearance when the plasma concentration is

kept level by a sustaining infusion over long periods has been demonstrated many times before. In Table III are PAH clearances in one of our subjects over the course of three and three-fourths hours

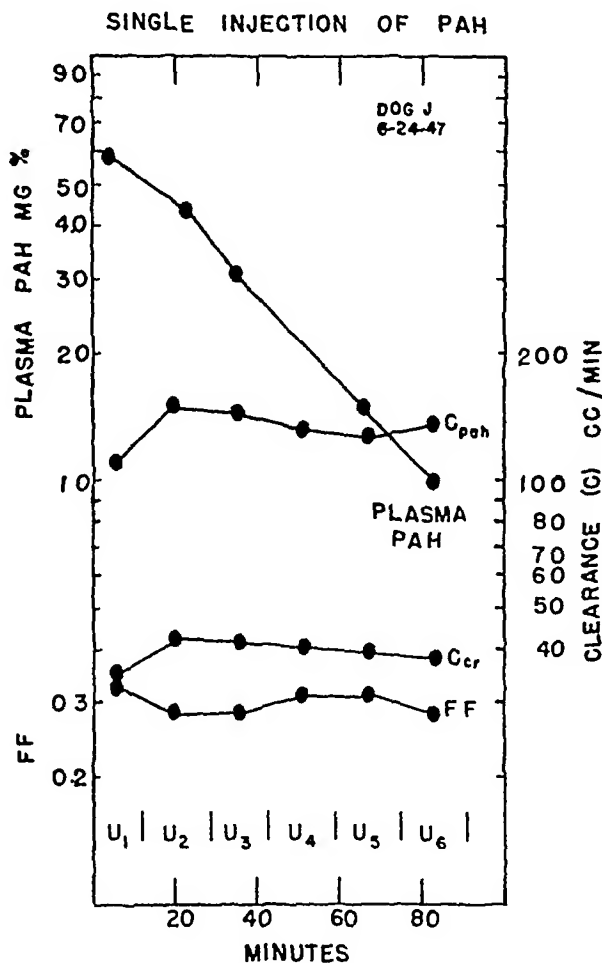


FIG 5 Constancy of PAH clearance ( $C_{PAH}$ ) in a dog with falling plasma concentration after single injection of 5 cc of 20% solution of PAH in 20 cc of saline.  $C_{cr}$  is clearance of creatinine.

Thus, exposure of the kidney to the same plasma concentration for many hours did not cause significant change in clearance.

The concept that the falling clearance of PAH in man with falling plasma concentration was caused by the venous plasma not being representative of the arterial blood was explored. If the depression of

clearance were due to a high venous concentration, arterial plasma determinations should reveal an arteriovenous difference, and the clearance calculated from arterial concentrations should be constant

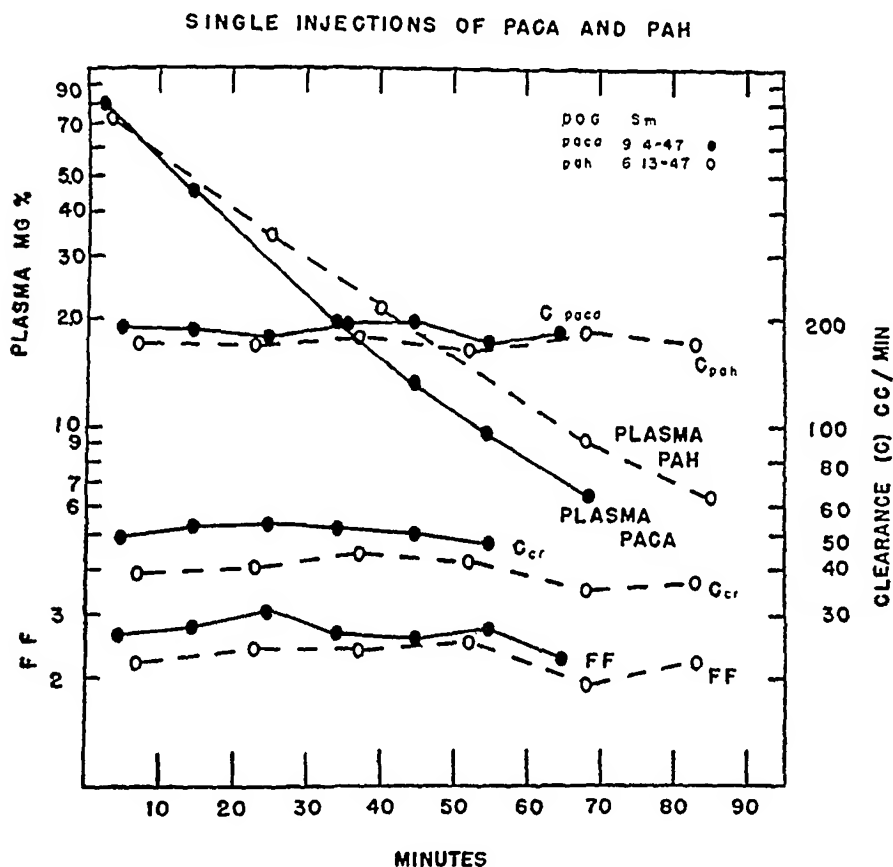


FIG 6 Single injections on different days in the same dog of 10 gram of PAH in 20 cc saline on 6-13-47, and 10 gram of PACA in 30 cc water at pH 7 on 9-4-47 show constancy of clearance with falling plasma concentrations over the same range for both substances

Symbols  $C_{PAH}$  is clearance of PAH  
 $C_{PACA}$  is clearance of acetyl PAH  
 $C_{Cr}$  is clearance of creatinine  
 FF is filtration fraction

In Figure 7 an arteriovenous difference in PAH plasma concentrations is demonstrated, but the falling concentration curves are parallel so that the "arterial" clearance falls. Thus, after a single injection of



PAH in man, the venous concentration is higher and proportional to the arterial plasma concentration. The dog shows a constant clearance of PAH determined from venous plasma (Figures 5 and 6), so that it is unlikely that an arteriovenous difference would explain the falling clearance obtained in man. Evidence that PAH is rapidly and freely diffusible from plasma to tissues will be presented later, indicat-

TABLE III

*Constancy of PAH Clearance Over Three and Three-fourths Hour Period of Continuous Intravenous Administration of PAH in Man*

Normal Male Subject, J B, Age 29, Surface Area 2.1 Sq M

Date 6/14/46

Continuous I V drip PAH Started at 8 00 A M

URINE COLLECTION PERIODS	PAH PLASMA CONC MID PERIOD	PLASMA CLEAR- ANCE PAH
	mg / 100 cc	cc / min
U <sub>1</sub> 8 15 to 9 17	1 02	608
U <sub>2</sub> 9 17 to 9 34	1 45	516
U <sub>3</sub> 9 34 to 9 55	1 42	407*
U <sub>4</sub> 9 55 to 10 14	1 40	518
U <sub>5</sub> 10 14 to 10 33	1 39	506
U <sub>6</sub> 10 33 to 10 53	1 35	579
U <sub>7</sub> 10 53 to 11 13	1 23	582
U <sub>8</sub> 11 13 to 11 34	1 19	535
U <sub>9</sub> 11 34 to 11 54	1 30	572

\* Small amount of urine lost

ing that it is unlikely that PAH would be stored in the tissues and released to the venous blood at a later time

*Observations on PAH Conjugated in Vivo ("PACA") and  
Para-acetylaminohippuric Acid (PACA)*

The behaviour of the clearance of PACA in dog and man was investigated. The solubility of PACA within the range of pH in urine

was determined. Clearances of PACA were determined after single injections in dogs. In Figure 6 the clearance of PACA is shown to be identical with the PAH clearance and is constant throughout the falling plasma concentration from 8 to 10 mg %. No self-depression of clearance occurred within this range. A larger dose of PACA was given in another experiment when the plasma concentration was raised

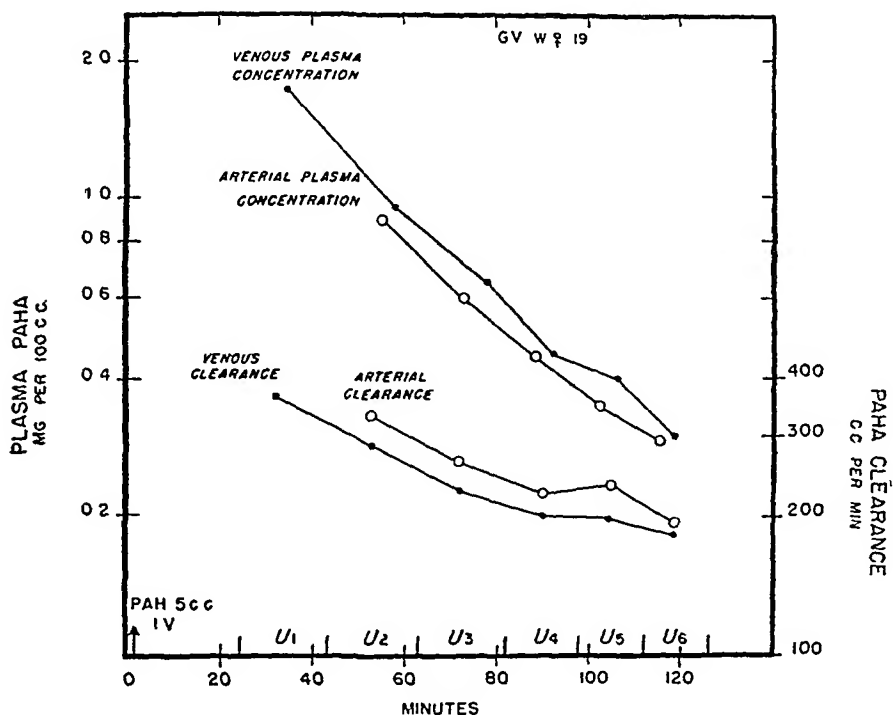


FIG 7 The arterio-venous difference in plasma concentration after a single injection of PAH, and the clearance calculated from both plasma curves. The arterial blood was obtained from an indwelling needle in the femoral artery and the venous blood from the arm vein. The clearances fall in a parallel manner.

to 28 mg % (Figure 8). Self-depression of clearance was present until the plasma concentration fell to 8.0 mg %, after which the clearance was maximal and constant. Thus, in the dog, PACA clearances like PAH clearances do not show depression or fall with a falling plasma concentration after either large or small single intravenous injections.

Similar data on a falling curve in a dog with an explanted kidney are given in Table IV with simultaneous determinations of renal vein

plasma concentration of PACA. The renal extraction of PACA was high throughout the whole range of plasma concentration. It appears, therefore, that PACA clearance in the dog measures the renal

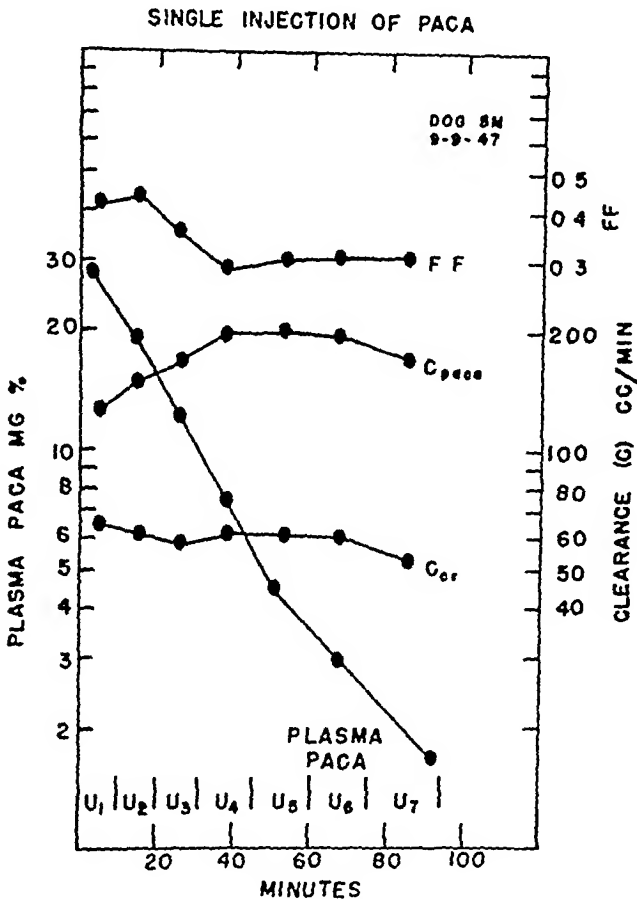


FIG 8 Single injection in a dog of 40 grams of PACA in 55 cc of water (pH 7). The ratio of creatinine clearance ( $C_{Cr}$ ) to PACA clearance ( $C_{PACA}$ ) is constant ( $F F$ ) between plasma concentration of 8.0 to 16 mg %. There is depression of PACA clearance above a plasma concentration of 8.0 mg % so that the filtration fraction is falsely elevated ( $F F$ ).

plasma flow as well as PAH clearance and that the clearance is independent of a changing or a previously high plasma concentration.

Determinations for the presence of "PACA" in plasma and urine after injections of PAH in the dog revealed no evidence of conjugation of PAH by the dog in any of the above experiments, nor was there any

evidence of de-acetylation of PACA, since no PAH could be found after injections of PACA

Because of the difference in behaviour of PAH clearances in dog and man after single injections, and because the dog *does not* conjugate PAH whereas man *does*, it seemed important to determine the amount of conjugated PAH in man and its mode of excretion. The results in Figure 9 show that as the PAH clearance falls the clearance of "PACA"

TABLE IV  
*The Renal Extraction of PACA in a Dog*

PERIODS	TIME	MID B PACA	C <sub>PACA</sub>	C <sub>CREAT</sub>	F F
	<i>Mins</i>	<i>mg %</i>	<i>cc /min</i>	<i>cc /min</i>	
1	11 5	11 7	87 1	27	31
2	23 0	7 3	108	31	28
3	33 5	4 7	126	28	224
4	50 5	3 25	112	30	27
5	69 5	1 80	122	32	26
6	85 5	1 11	107	32	298
7	104 0	76	88 5	30	34
PERIPHERAL PACA		RENAL VEIN PACA		EXTRACTION	
<i>mg %</i>		<i>mg %</i>		<i>%</i>	
9 7		1 9		80	
4 8		1 1		77	
1 13		12		90	
67		10		85	

Dog Gerry. Peripheral blood obtained from jugular vein. Renal vein blood obtained by direct puncture of renal vein in an explanted kidney preparation. Single injection of 1 gram of PACA in 20 cc water at pH 7.

Explantation of kidney performed by Dr. Willard Goodwin, Resident Urologist.

remains higher. Fluctuation in the calculated clearance of "PACA" is seen because the plasma concentration of this substance is low and is obtained by difference between the PAH and total concentrations. Thus, the accuracy of the plasma "PACA" determination is questionable. Nevertheless, it seems significant that the clearances of conjugated PAH are always higher than those of PAH.

The proportions of PAH present in the urine during the observations in Figure 9 are given in Table V. As the rate of excretion and

the plasma concentration of PAH fall, there is an increased proportion of conjugated PAH in the urine

In view of the fact that conjugation of PAH may have been responsible, at least in part, for the falling clearances, we have made some

### SINGLE INJECTION FREE AND TOTAL CLEARANCE

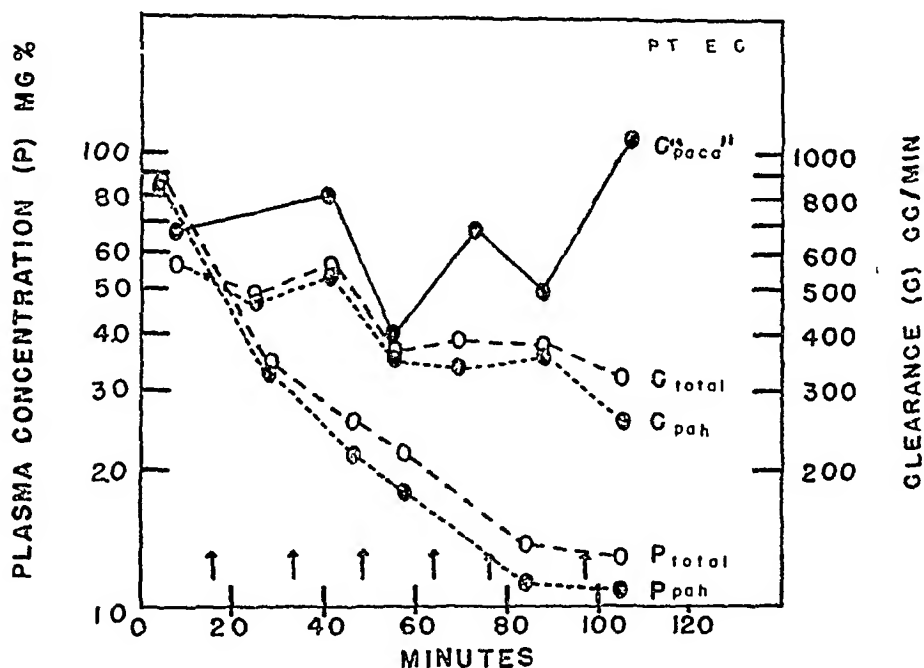


FIG 9 The plasma concentration and clearances of PAH and "PACA" (PAH conjugated in vivo) after a single injection of PAH in a normal subject. The clearance of "PACA" is always higher than the clearance of PAH which steadily falls. Calculation of clearance from total concentrations (PAH + "PACA") also yields a falling curve. See Table V for the relative amounts of PAH and "PACA" in the urine in this experiment.

preliminary observations on the behaviour of PACA clearances after single injections. In Figure 10, 11 and 12 are observations, after single injections, showing that the clearance of PACA does not show the progressive fall encountered previously with PAH clearances within the range of plasma concentration of 7 to 10 mg% (Compare with PAH clearance in Figure 8). Variations in PACA clearance

were accompanied by proportionate changes in the clearance of inulin so that the filtration fraction ( $F/F$ ) remained nearly constant. The constant  $F/F$  confirms the fact that there is no progressive depression of PACA clearance. High filtration fractions were encountered in the cases with hypertension or cardiac failure.

TABLE V

*The Relative Concentrations of "Free" PAH and Total PAH (Free + Conjugated) in Urine Following Single Intravenous Injection of PAH in Man*

Normal Male Subject, E. C., Age 21, Surface Area 1.83 Sq M

Date 12/23/47

PAH—5 gms I.V. between 9:33 A.M. and 9:46 A.M.

URINE COLLECTION PERIOD	PAH (UV)	TOTAL PAH + CONJUGATED PAH (UV)	CONCENTRA- TION RATIO	PAH PLASMA CONC. MID- PERIOD
	mg/min	mg/min	PAH/Total	mg/100 cc
U <sub>1</sub> 10 03 to 10 18	4 10	43 0	95.5%	7 3
U <sub>2</sub> 10 18 to 10 36	1 73	18 8	92.0%	3 7
U <sub>3</sub> 10 36 to 10 51	1 30	16 0	81.4%	2 43
U <sub>4</sub> 10 51 to 11 06	6 57	8 21	80.0%	1 85
U <sub>5</sub> 11 06 to 11 19	4 89	6 79	72.0%	1 46
U <sub>6</sub> 11 19 to 11 40	3 97	5 10	77.9%	1 12
U <sub>7</sub> 11 40 to 11 56	2 75	4 01	68.5%	1 08

See Figure 10 for the behaviour of the clearances of PAH and conjugated PAH.

After injections of PACA into man, no "free" PAH was found in the plasma or urine. Thus de-acetylation of PACA does not occur in man.

### *Concerning Priming Doses*

It is possible that the temporary elevation of plasma concentration by the priming dose to levels far above the ultimate level to be maintained by the constant infusion, may cause a falling plasma level during the first periods of clearance determination, with resultant depres-

# SINGLE INJECTION OF PACA

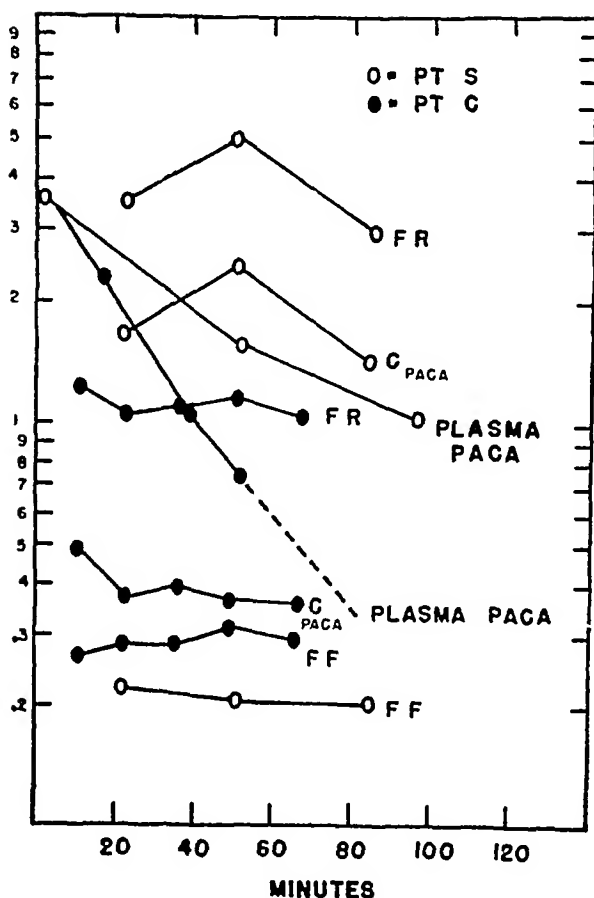


FIG 10 The constancy of filtration fraction (F F) in two children with falling plasma PACA concentrations after single injections of thiosulfate or inulin and PACA

*Patient C* (●) Diagnosis Coarctation of aorta with hypertension

Weight 98.5 lbs, Height 58 inches

Single injections of 10 grams of sodium thiosulfate in 100 cc water with 2.35 grams of PACA in 100 cc water at pH 7

*Patient S* (○) Diagnosis Coarctation of aorta after surgical resection of aortic isthmus by Dr Alfred Blalock Blood pressure normal

Weight 37.7 lbs, Height 42.3 inches, Age 5 years

Single injections of 3 grams of inulin in 30 cc saline and 1.0 gram of PACA in 25 cc water at pH 7

While the filtration rate and clearance of PACA vary, particularly in patient S, there is constancy of the filtration fraction (F F). Marked variations in clearances with constant F F are probably due to inaccurate urine collections. There is a constant relation of the clearance of PACA to the clearance of thiosulfate or inulin with a falling plasma PACA concentration over the range of 3.6 to less than 1.0 mg % in both patients. The higher F F (27 - 30) is found in the child with hypertension. Urine was collected by catheter.

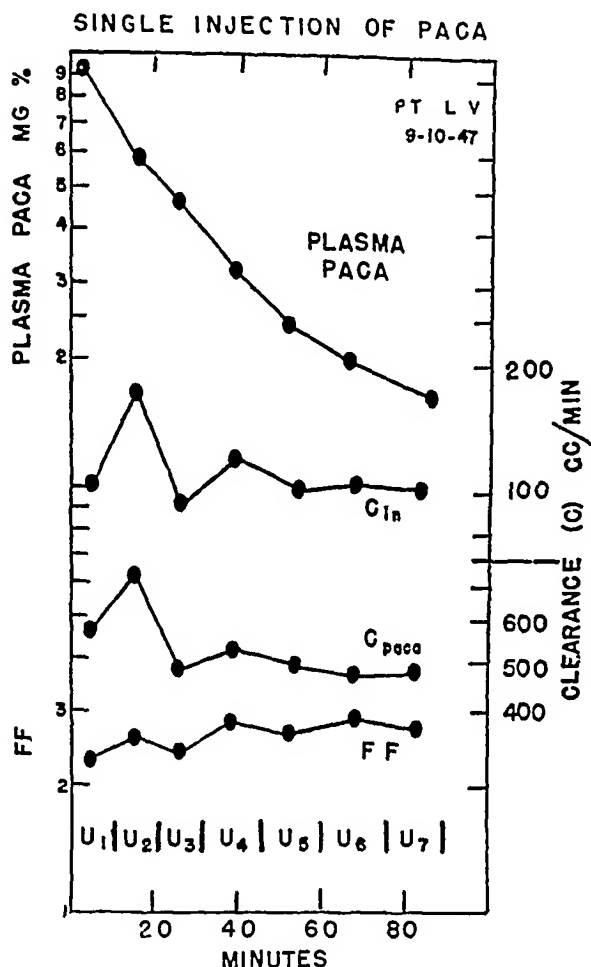


FIG 11 Patient L V, Age 18, Weight 141 lbs, Height 68 inches, diagnosis hypertension Single injections of inulin 10 grams in 100 cc saline and 50 grams PACA in 100 cc water at pH 7

The marked variations in clearance values in the first three periods are probably due to difficulty encountered in urine collection The F F was within the range of 23 to 29 as the PACA plasma level fell from 9.28 to 1.65 mg % Apparently there was no significant self-depression of the PACA clearance at a plasma concentration of 5 to 9 mg %, since the F F was not elevated Depression of PACA clearance should raise the F F

sion of clearance, such as is demonstrated with falling plasma levels following single injection with no sustaining infusion, or may cause a depression of clearance due to temporary exposure to higher concentrations



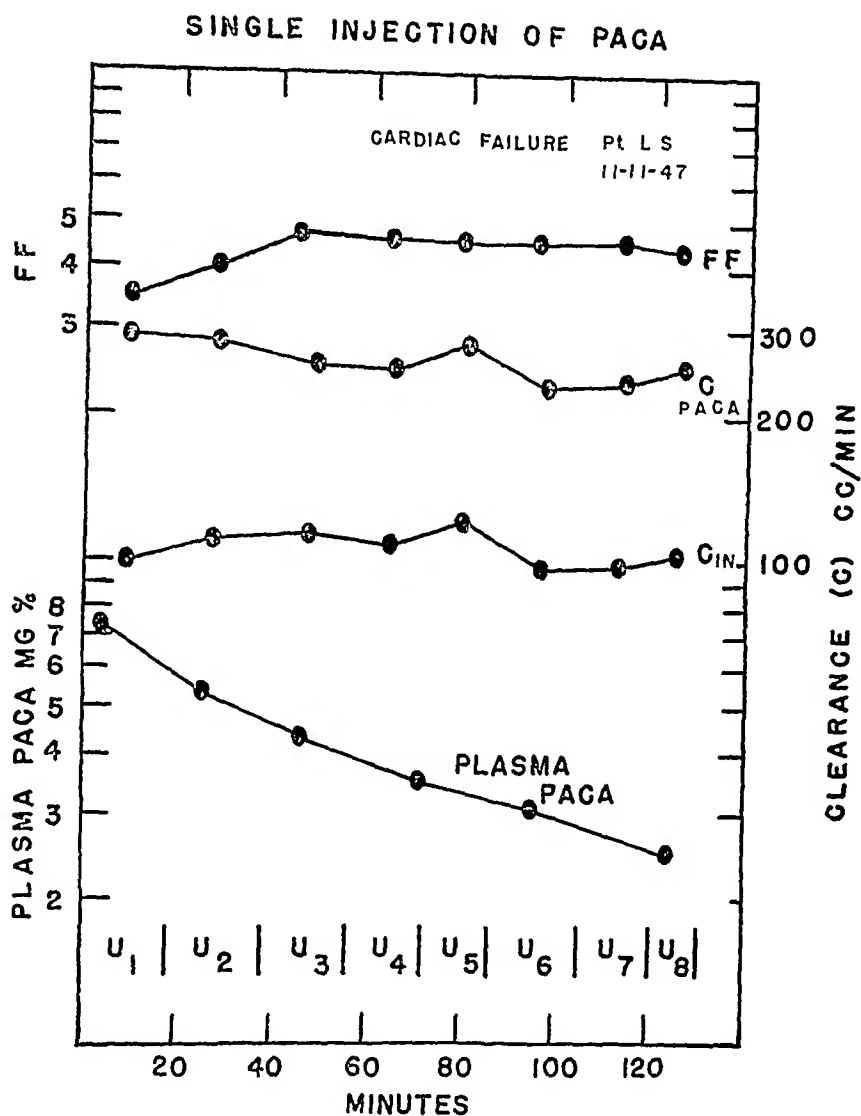


FIG 12 Patient L S, Age 38 Cardiac failure due to rheumatic heart disease with murmurs of mitral stenosis and insufficiency and aortic insufficiency with peripheral edema, orthopnea and elevated venous pressure Patient was propped up in a cardiac bed throughout the examinations

Single injections of 10 grams of inulin in 10% solution and 5.0 grams of PACA in 50 cc water at pH 7 Urine collected by catheter

The filtration fraction is abnormally high, but after the first three periods it is constant with the plasma concentration of PACA falling from 4.33 mg % to 2.48 mg %

Thus, it seemed important to determine if priming doses are really necessary if constant plasma levels are desired. The first step in approaching this problem was to derive a theory which would have general application to the relationships between a constant rate of infusion (IV), the apparent volume of distribution ( $V_c$ ), the renal clearance (C) and the plasma concentration (P) of a substance such as PAH or PACA.

These symbols are used in the following derivation

(IV) = mg/min infused at a constant rate

C = cc/min of plasma cleared

$V_c$  = cc of body fluid in which substance is apparently dissolved

(UV) = mg/min excreted

P = mg/cc concentration in plasma or in  $V_c$

M = total mg of PAH in body at any time

t = time after beginning of constant infusion

The assumptions are made that loss of this substance from the body by routes other than the kidney is negligible compared to the rate of excretion by the kidney, and that the apparent volume of fluid in which it is dissolved remains constant.

The change in M with time will be the result of the sum of the rate of infusion and the rate of excretion

$$(1) \frac{dM}{dt} = (IV) - (UV)$$

The plasma concentration at any time will be

$$(2) P = \frac{M}{V_c}$$

$$(3) \text{ thus } M = PV_c$$

Substituting  $PV_c$  for M in equation (1)

$$(4) \frac{dPV_c}{dt} = (IV) - (UV)$$

Since  $V_c$  is constant we may rearrange as follows

$$(5) \frac{dP}{dt} = \frac{(IV)}{V_c} - \frac{UV}{V_c}$$

Since by definition  $\frac{(UV)}{P} = C$  or  $(UV) = CP$

$$(6) \frac{dP}{dt} = \frac{(IV)}{V_c} - \frac{C}{V_c} P$$

Finally rearranging before integration,

$$(7) dp - \frac{(IV)}{V_c} dt + \frac{C}{V_c} P dt = 0$$

Integration leads to the final equation

$$(8) P = \frac{(IV)}{C} [1 - e^{-(C/V_c) t}]$$

From inspection of this equation, it is apparent that  $\frac{(IV)}{C}$  is the plasma concentration which is approached with time and that the plasma concentration attained at any given time will be directly proportional to the rate of infusion (clearance and  $V_c$  remaining constant). This is illustrated in Figure 13.

The shape of the plasma concentration curves with changes in  $V_c$  or in clearance ( $C$ ) are shown in Figure 14 and Figure 15 respectively. For these illustrations, values for clearance and rate of infusion were chosen which are in the range encountered in the determination of clearance by the constant infusion technique. The illustrations are theoretical curves derived by substitution of arbitrary reasonable values for  $(IV)$ ,  $(C)$  and  $(V_c)$  in equation (8).

In the relationships shown in equation (7), the only factor which is not directly determinable experimentally is the apparent volume of distribution ( $V_c$ ). This can be calculated from experimental data. In Figure 16 there are two experimental curves of plasma level of PAH

after starting a constant infusion The apparent volume of distribution of PAH was 4000 to 5000 cc in these subjects

### CONSTANT CLEARANCE AND VOLUME VARIABLE INFUSION RATE

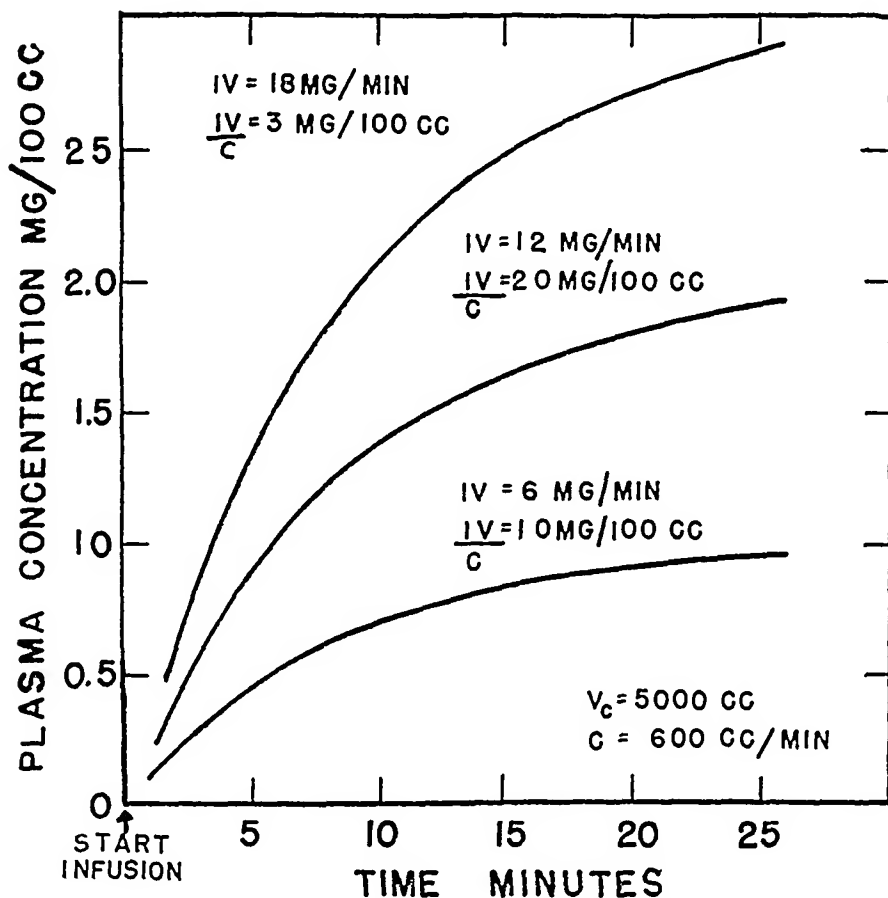


FIG 13 Theoretical curves for plasma concentration of PAH or PACA derived from Equation 8 using various infusion rates with a constant clearance and constant apparent volume of distribution

Thus, the plasma concentration, after beginning a constant infusion of PAH, follows the curve which we would expect if it were distributed in approximately five liters of body water. From the practical standpoint, both the theoretical analysis (Equation 8) and the experimental

facts serve to show that 20–30 minutes after starting a constant infusion the plasma level will have reached a maximum and constant value, and the time necessary to reach the maximum value will be in-

### CONSTANT INFUSION RATE AND CLEARANCE VARIABLE VOLUME

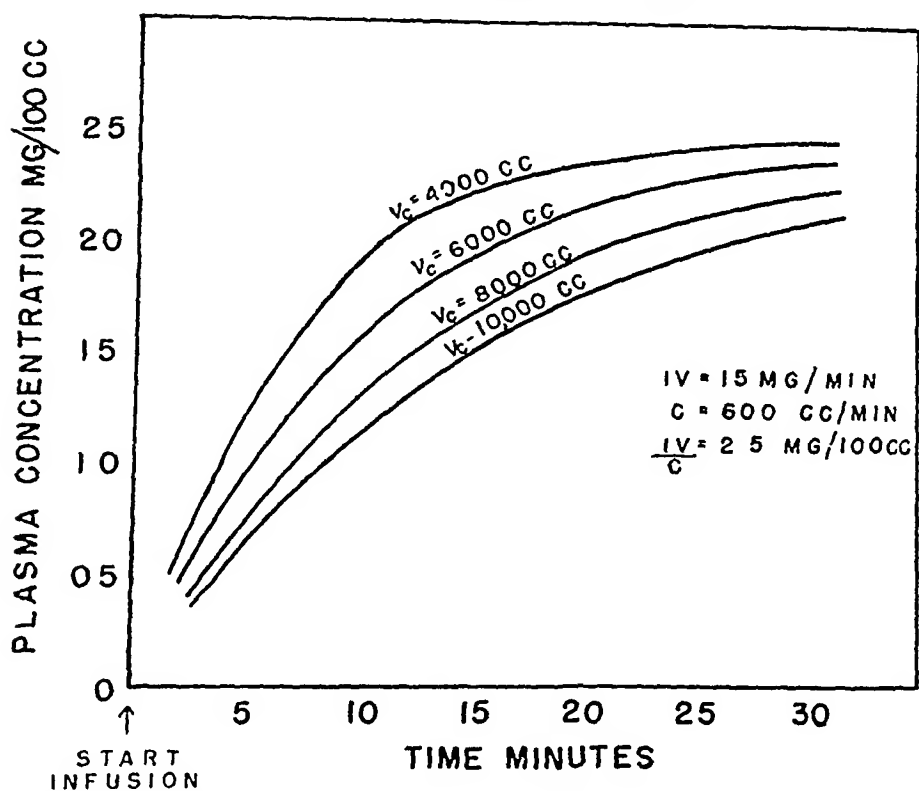


FIG 14 Theoretical curves for plasma concentration of PAH or PACA derived from Equation 8 using various values for the apparent volume of distribution with constant rate of infusion and constant clearance

dependent of the value for rate of infusion and clearance, provided they are constant

Thus, the practice of giving "priming" doses of a substance such as PAH to be followed by a constant maintenance infusion is unnecessary. Since it usually requires 20–30 minutes for equilibration of substance such as PAH after the initial flooding of the circulation with the prim-

ing dose, the constant infusion by itself will accomplish the same constant blood level in the same time. Furthermore, there are other

### CONSTANT INFUSION RATE AND VOLUME VARIABLE CLEARANCE

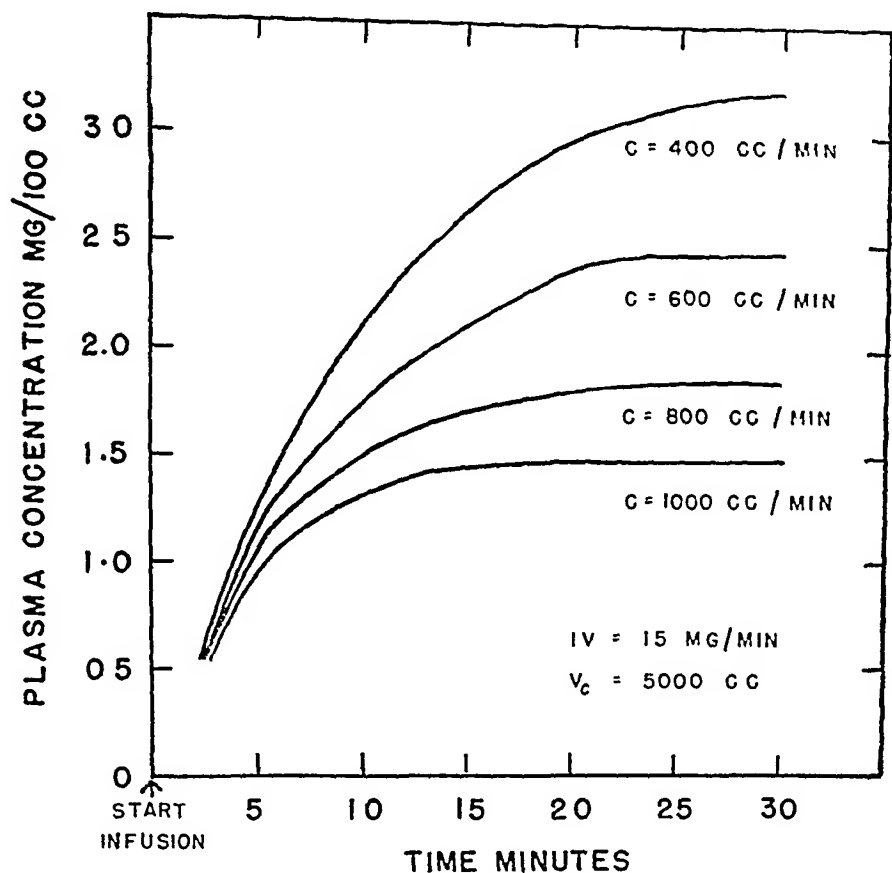


FIG 15 Theoretical curves of plasma concentration of PAH or PACA derived from Equation 8 calculated using various clearance values with a constant rate of infusion and constant apparent volume of distribution

objectionable features to priming doses. Side reactions such as flushing, chilliness and sweating usually occur during the injection of the initial concentrated solution used for a priming dose. These can be avoided by omitting the priming dose entirely in experiments during which a constant plasma level of PAH or of PACA is desired.

## CONSTANT INFUSION

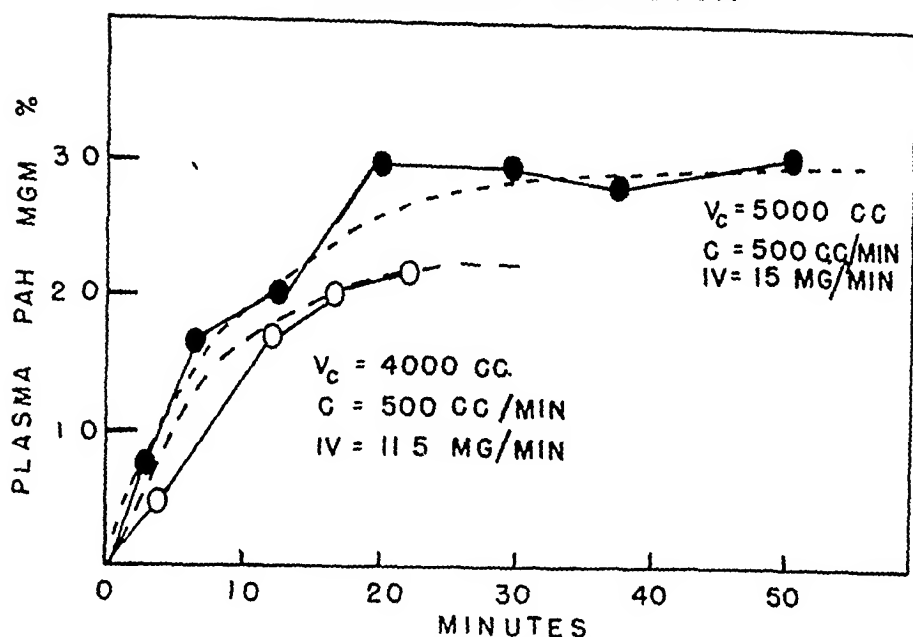


FIG 16 CONSTANT INFUSION OF PAH WITHOUT A "PRIMING" DOSE

The dotted curves are obtained from Equation 8. By substitution of the experimental values for  $C$  (IV) and  $P$ , the values for  $V_c$  were obtained, giving the curves which best fit the experimental data. The constant infusion was started at time  $\approx 0$ . The solid lines are the plasma concentrations found in the two experimental subjects.

## DISCUSSION

In the earlier studies with phenol red on the relationship of clearance to plasma concentration, single injections were used. Shannon, working with dogs (6), and Goldring, Clark and Smith with humans (7), gave single small and large injections of phenol red and concluded that the clearance was completely reversible. The clearance returned to the same high level with any dose used.

However, phenol red clearance was soon abandoned in favor of diodrast clearance as a measure of renal plasma flow, since diodrast yielded a higher clearance. The relationship of diodrast clearance to plasma concentration in man was described by Smith et al (3), and this description is the basis of the relationship given in Figure 1. It appears, however, that no experimental facts were recorded showing that there is a constant maximal clearance range in man after single injections of

diodrast, or that self-depression of diodrast clearance is completely reversible

Data on man after single injections of diodrast were recorded by White, Findley and Edwards (8). No comment was made on the fact which is apparent from their data that the clearance of diodrast showed a tendency to fall throughout the range of plasma concentration where the clearance was thought to be maximal and constant. Also, the clearances obtained with constant infusion at low plasma concentrations seem to be higher than the clearances after single injections.

The reason for the falling clearance and lack of reversibility of diodrast clearance after self-depression is not clear. Factors such as delay time, differences in arterial and peripheral venous plasma concentration, and changing urine flow would be unlikely causes of such large errors in the clearance determination. The facts that phenol red and PACA in man, and phenol red, PAH and PACA in the dog, yield reversible clearances and a constant maximal range after single injections make it likely that our findings are peculiar to diodrast and the human kidney, rather than to secondary circulatory or timing relationships. It is possible that diodrast is altered or conjugated in the body in a manner such that it would not be detected by the methods of determinations, which depend solely on the amount of iodine excreted. Whether different products of diodrast appear in human urine is now being investigated.

In the first studies on the excretion of hippuric acid derivatives by Smith (9, 10), it was reported that PAH in human urine was partly conjugated and that the clearances of PAH and PACA were equal both in dog and in man. Our finding, that there were increasing amounts of conjugated PAH in the urine with a falling plasma level, led us to suspect that either the excretion of the conjugated product was competing with PAH or that PAH was being conjugated by the kidney. The presence of a higher proportion of conjugated PAH in the urine than is present in plasma suggests that either the excretion of conjugated PAH takes precedence over the excretion of PAH or that the PAH is conjugated by the kidney.

It would be of great interest to determine the amount of PAH and "PACA" present in arterial and renal venous plasma simultaneously with the amount in urine. Demonstration of complete extraction of



PAH alone in renal vein plasma does not reveal whether there is any PACA present or if all PAH is present in the urine unmodified. Certainly it would be more desirable to use a substance which is not modified in the body and avoid the possibilities of interfering secondary substances.

Our findings emphasize that careful evaluation of many factors in the technique must be made in any experiment. With a perfectly constant plasma level, the proportion of conjugated PAH excreted or formed in the kidney may be small and constant. However, a change in plasma concentration may result in a change in the proportion of conjugated PAH in the urine and a change in clearance which is not a change in renal plasma flow. It should be emphasized again that there appears to be no definite data as to what chemical forms of diodrast and PAH appear in human urine after intravenous injection. When one instantaneous plasma determination is applied to the calculation of a clearance period lasting many minutes, absolute constancy of plasma concentration is essential for accuracy. The maintenance of a perfectly constant infusion is therefore necessary.

With a falling plasma concentration after a single injection, one eliminates the difficulty of maintaining a perfectly constant infusion, and one can describe the course of the plasma level accurately since it follows a consistent curve. However, other difficulties are introduced by using a falling concentration. One must limit the length of the observations so that the plasma concentration remains within the range of maximal clearance. Also, there is the possibility of arteriovenous differences in plasma concentration. Such differences would be more likely to cause error when the plasma concentration has fallen to low levels, if the arterial is not proportional to the venous plasma concentration.

Since it is desirable to determine clearances on the same plasma which reaches the kidney, namely arterial plasma, we are now investigating a method for analysis of "arterial" plasma obtained from finger blood. Enough plasma (0.2 cc) can easily be obtained from blood from a finger tip puncture. It may be possible then to determine clearances after a single injection of PACA, eliminating any error due to arteriovenous differences in plasma concentration and obtaining

more frequent samples without venapunctures and loss of greater quantities of blood

#### SUMMARY

1 Constant plasma clearances were obtained with falling plasma concentrations after single injections of PAH and PACA in dogs and with PACA in man

2 Falling clearances were obtained in man, with falling plasma concentrations of diodrast and PAH after single injections, and with plasma concentrations sustained by infusions after temporary elevation of plasma level

3 Conjugated PAH appears in increasing proportion to PAH in the urine with falling plasma concentration, and the clearance of conjugated PAH becomes higher than the clearance of PAH

4 The many factors which could produce invalid clearance measurements of renal plasma flow are discussed. Two factors may, under some circumstances, be important: modification of the injected substance, a difference between arterial and venous plasma concentration

5 A general theory is derived of the relationship between rate of infusion, plasma concentration, and plasma clearance of a substance excreted at a rate proportional to the plasma concentration

6 If a constant plasma concentration is desired, a "priming" dose before the sustaining infusion is unnecessary

7 A method of preparation and determination of PACA is described

Dr E K Marshall and Dr K Blanchard of the Department of Pharmacology have given valuable advice and criticism

We are indebted to Dr Margaret Merrell, Associate Professor of Biostatistics, School of Hygiene and Public Health, Johns Hopkins University, for aid with the theoretical analysis

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# AN UNUSUAL CASE OF SQUAMO-BASAL CELL CARCINOMA

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It is well known that basal cell carcinoma is not a morphologically closed entity, there are transitional forms between the histologically pure basal cell carcinoma and the prickle or squamous cell carcinoma (1) Basal cell tumors in which there is some differentiation towards squamous cell carcinoma display a greater degree of malignancy than those in which no such differentiation is present (2, 3) Moreover, these transitional forms, or squamo-basal cell carcinomas, in the majority of cases are relatively resistant to roentgen-ray and radium treatments as compared with pure basal cell tumors (3) The purpose of the present report is to describe a squamo-basal cell carcinoma of extreme malignancy and extraordinary morphology

A sixty eight year old white man was admitted to the private surgical service of The Johns Hopkins Hospital (History No 255034) on June 14, 1946, complaining of intermittent episodes of left upper quadrant abdominal pain of two months duration

There had been several previous admissions to the hospital—in June, 1923 for tonsillectomy and adenoidectomy, and in March, 1942 for appendectomy Of greater interest, however, was a series of admissions that started on February 10th, 1941 when a chalazion was removed from the inner third of the left upper eyelid at the Union Memorial Hospital On microscopic examination it was diagnosed "Basal Cell Epithelioma " On April 29th, 1943 at the Johns Hopkins Hospital a small nodule was removed from the left upper eyelid at the same site as the chalazion excised in February, 1941 On microscopic examination it was diagnosed "Squamous Cell Carcinoma " On October 1st, 1943 at the Union Memorial Hospital almost the entire inner half of the left upper eyelid was removed with a portion of tarsal cartilage No pathological report on this tissue is available A plastic repair on the eyelid was done On April 6th, 1944 at the Union Memorial Hospital a small nodule at the site of the scar of the last operation was excised together with a swollen preauricular lymph node Both specimens were reported as showing a similar type of tumor and the diagnosis was "Carcinoma, skin appendage, upper eyelid " On September 17th, 1944 at the same hospital a growth was removed from the outer half of the left upper eyelid and a swollen lymph node was excised

from the left side of the neck The pathological report was "Carcinoma, epidermoid, basal cell, with metastasis to lymph nodes "

During the period from July, 1941 until April, 1946 the patient received large amounts of roentgen and radium treatment directed first to the lesion on the left upper eyelid and later to the left neck and sub-maxillary region Under this therapy he developed hypothyroidism in early 1945 so that thyroid extract, one grain per day, had to be administered

The family history was entirely non-contributory The personal history revealed a life long tendency for eczematoid eruptions to occur particularly on the skin of the face

The present illness began in April, 1946 when there occurred episodes of left upper quadrant abdominal pain, bearing no relationship to meals or any other recognizable factor At about the same time he began to suffer from constipation These symptoms became worse and anorexia developed Then he noticed progressive and severe limitation of stomach capacity, liquid foods being tolerated more easily than solid There was one episode of vomiting and several periods of severe nausea The patient had become weaker and had lost about ten pounds in weight in the two months prior to admission

On admission to hospital the temperature was 97.6°, the pulse 80, respirations 20 and blood pressure 100/60 The patient was an elderly male in no apparent distress His skin showed evidence of recent weight loss The left upper eyelid showed the result of excision of portions of it and subsequent graftings No nodules were felt in it Under the angle of the left jaw there was a hard mass (size not mentioned) which felt like a lymph gland, discrete but attached to the deep tissues The skin was indurated over the left posterior cervical region The thyroid could not be palpated There was no generalized lymph node enlargement, but there were several subcutaneous nodules, each measuring roughly about 7 mm in diameter, over the thorax and abdomen The lungs were clear on percussion and auscultation The heart was normal The abdomen was relaxed and there was no tenderness or muscle spasm The liver was palpable two fingers breadth below the costal margin The spleen could not be felt In the epigastrium there was resistance, but no well defined mass could be felt Rectal examination revealed a diffusely enlarged and moderately firm prostate Neurological examination was negative

Examination of the blood showed Hbg 11 gm, WBC 6,200 The urine was negative The blood serological test for syphilis was negative No organic lesion could be demonstrated by a barium enema A gastrointestinal series was done, the lesser curvature of the stomach was very rigid and no peristaltic waves were seen going through that portion of the stomach This was interpreted as suggesting the presence of an infiltrative lesion in that region

The clinical diagnosis was carcinoma of the stomach

On June 17th, 1946 an exploratory laparotomy was performed The stomach was described as being a solid mass of tumor and operative removal of its was con-

sidered impossible. A biopsy of an enlarged omental lymph node was made. The pathological diagnosis was "Squamo-basal cell carcinoma."

Postoperatively the patient's condition gradually became worse and he died on August 1st, 1946.

The autopsy (No 20074) was performed 12 hours after death. The report is abbreviated, but no pertinent observations are omitted.

The body is that of a well developed, poorly nourished, white adult male, measuring 169 cm in length and weighing 59.5 kg. The superficial lymph nodes are not palpable except in the cervical and axillary regions, in the latter location a firm nodule about 2 cm in diameter is felt. On the left upper eyelid there is a small scar causing the eyelid margin to be irregular. No skin lesions are described.

There is about 1500 cc of clear fluid in the peritoneal cavity. The peritoneal surfaces are everywhere studded with small yellowish-white tumor nodules each measuring about 5 mm in diameter. These nodules extend in linear fashion along the mesenteric attachment of the entire small intestine. In the subdiaphragmatic and pelvic regions they are more numerous and larger in size. In the left hypochondrium the stomach and transverse colon are bound together by tumor tissue. The peripancreatic and para-aortic lymph nodes are all enlarged and firm, about the origins of the renal arteries there are lymph nodes measuring as much as 2 cm in diameter and having bulging granular grayish-white cut surfaces.

Each pleural cavity contains about 500 cc of clear fluid. There are no adhesions. The pericardial cavity is normal. The site of the thymus is occupied by fatty tissue, there is no mass in this region. The mediastinal lymph nodes are enlarged, especially at the bifurcation of the trachea where several of them, each measuring about 1 cm in diameter, are seen, they are firm in consistency and on section present a bulging grayish white cut surface with small areas of anthracotic pigmentation.

The heart weighs 350 grams. The valves are normal. The orifices of both coronary arteries are narrowed and there is sclerosis and calcification of all the coronary arteries with marked eccentric narrowing of their lumina. Two tumor nodules are seen in the myocardium, one of these measures about 3 mm in diameter and lies in the anterior wall of the left ventricle towards its base, the other forms a greyish pearly area about 1 cm in diameter high up in the interventricular septum.

The pleural surfaces of both lungs are smooth. The lungs are air-containing. The bronchial mucosa is normal everywhere.

The liver is firmly adherent to the underlying stomach. The cut surface shows slight central atrophy and congestion and a single tumor nodule about 1 cm in diameter is seen. The gall bladder is thin walled and contains numerous small faceted stones.

The spleen weighs 100 grams. The capsule is thickened in one area.

The oesophagus is normal. The stomach is adherent to the liver, pancreas and a portion of the transverse colon. Its mucosa is flat and there is no ulceration. The stomach wall is infiltrated with greyish white tumor tissue especially in the region of the fundus where the wall measures about 1 cm thick. The duodenum

is normal The mucosa of the jejunum and upper ileum is normal In the lower ileum two or three greyish white tumor nodules are seen projecting from the mucosal surface, the mucosa over these areas is ulcerated so as to form a rounded crater in each nodule The mucosa of the large intestine is normal The mesenteric lymph nodes are not enlarged

The peripancreatic lymph nodes are replaced by tumor and many tumor nodules are seen in the pancreas itself

The medulla of the left adrenal is replaced by a greyish white nodule of tumor tissue measuring about 1 cm in diameter In the medulla of the right adrenal a smaller nodule measuring about 5 mm in diameter is seen

The kidneys are normal except that the pelvis and calyces of the left kidney are slightly dilated The right ureter is delicate and patent, the left ureter and the inferior portion of the left renal pelvis show thickening of their walls by invading tumor tissue—the left ureteral wall throughout its length measures about 5 mm in thickness The ureteral openings into the bladder are patent The prostate is slightly enlarged and on section several small firm nodules are seen in its right lobe

The thyroid is very small and difficult to identify grossly The paratracheal lymph nodes are slightly enlarged and are invaded by tumor The tongue, pharynx, larynx, and trachea are normal The tonsils are not present

The bone marrow of the ribs appears normal In the lumbar vertebral marrow there are pale yellowish areas suggesting tumor metastases

The brain and hypophysis are grossly normal

Study of the microscopical sections from the organs show little apart from invasion by tumor There is atrophy and scarring of the thyroid gland, probably caused by irradiation The pancreas shows dilated ducts, atrophy and scarring due to obstruction of its duct system by tumor In the anterior lobe of the hypophysis there is a poorly preserved adenoma There is lobular pneumonia in the lungs and a small adenoma is seen in the prostate

The tumor invades the following tissues cervical, mediastinal and para-aortic lymph nodes, peritoneum, stomach, liver, pancreas, capsule of spleen, adrenals, lung, left ureter and renal pelvis, lower ileum, myocardium and vertebral bone marrow

The remarkable feature of this tumor is that it is composed, in places, of round cells with deeply staining nuclei and scanty cytoplasm These cells grow in the most diffuse manner, suggesting the appearance of a lymphosarcoma (Fig 1) In all of the sections there are tiny round areas of squamous cells with keratinization and pearl formation In some places these areas lie in the midst of the diffuse growth of round cells so that the tissue bears a striking resemblance to thymic tissue, the tiny, round squamous foci suggesting Hassall's bodies (Fig 2) In other places the pearls are surrounded by spindle shaped cells with hyperchromatic nuclei and scanty cytoplasm These cells tend to

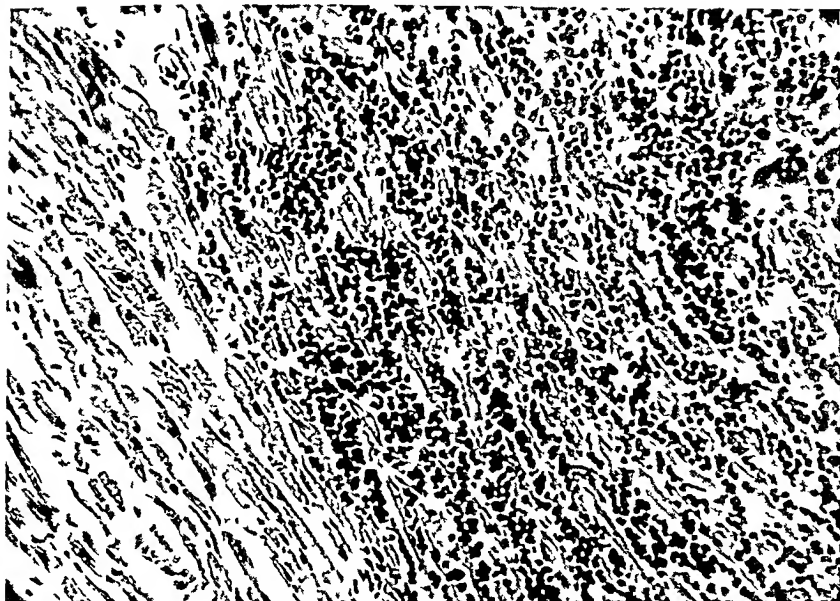


FIG 1 TUMOR IN MYOCARDIUM NOTE TYPE OF CELL AND DIFFUSE INFILTRATION SUGGESTING LYMPHOSARCOMA

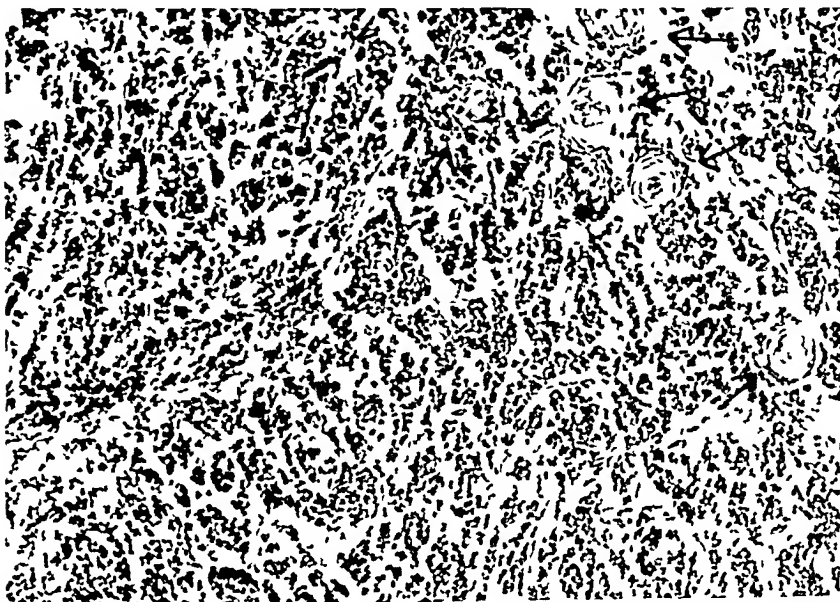


FIG 2 TUMOR IN CONNECTIVE TISSUE AROUND RENAL ARTERY DIFFUSE GROWTH OF ROUND CELLS WITH SQUAMOUS FOCI (ARROWS) SUGGESTING THYMIC TISSUE



cling together rather than to be diffusely scattered. There is no peripheral palisading of cells around these groups. It is in these latter areas that the similarity to squamo-basal cell carcinoma is evident. No intercellular bridges are present between any of the cells.

Review of the series of biopsies taken from the left upper eyelid and involved lymph nodes during the patient's life makes it reasonably clear that the tumor originally arose from the epithelium of the face and later metastasized widely. The first section removed from the eyelid over five years before death shows a squamo-basal cell carcinoma in which the basal elements are closely grouped together and surrounded by a definite palisade of cells. The subsequent biopsies show a gradual transformation into a tumor in which the cells, for the most part, no longer form bands or coherent masses but infiltrate not infrequently as separate cells (Fig 1) which have round or elongated hyperchromatic nuclei and very little cytoplasm.

#### DISCUSSION

This tumor ran a much more malignant course than is seen in basal cell tumors, and displayed a marked insensitiveness to irradiation.

Microscopically, although the tumor seemed to fall into the group of squamo-basal cell carcinomas, its structure was decidedly unusual. The cells, for the most part, did not cling together to form nests and strands and distinct groups as in the usual basal cell carcinoma. On the contrary they tended to infiltrate diffusely, often as single cells, and indeed in many microscopic fields the tumor could easily be mistaken for a lymphosarcoma (Fig 1) or, in areas where there were foci of squamous cells with keratinization, for thymic tissue (Fig 2).

As far as can be determined, no similar case has been reported. However, in his description of "transitional cell carcinomas", Ewing (4) makes the statement "In very active cases of this type, the cells may lose most or all of their epithelial characters, and the tumor may grow diffusely and be difficult to distinguish from lymphosarcoma or lymphoepithelioma." But these carcinomas generally fail to show squamous cells (4).

#### SUMMARY

This report describes a case of extremely malignant squamo-basal cell carcinoma, which was remarkably peculiar in that the basal cell

elements, in many places, grew in a diffusely invading manner suggesting the morphology of a lymphosarcoma. Associated with the basal cells, there were minute, globoid squamous cell formations.

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# GAUCHER'S DISEASE WITH THROMBOCYTOPENIA, AN INSTANCE OF SELECTIVE HYPERSPLENISM

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## A CASE REPORT

In recent years great interest has been manifested in the concept of hypersplenism, a term denoting reduction of the formed elements of the peripheral blood in association with splenomegaly. This effect has been attributed by some to altered phagocytic function of the reticulo-endothelial cells of the spleen, and by others to the influence of a possible humoral agent of splenic origin on the bone marrow when splenic hypertrophy occurs. Hypersplenism may be selective or panhematocytopenic, i.e., depression of any one of the formed blood constituents or any combination of these elements may be observed. Recently a case was observed by us in which selective hypersplenism (thrombocytopenia) was associated with the splenomegaly of Gaucher's disease.

## CASE REPORT

S D, a 32 year old Alabama housewife, was admitted to the Johns Hopkins Hospital 7/25/47, on the service of Dr J E Howard, complaining of a mass in the abdomen which had been known to be present for 2½ years. The family and past histories yielded no material pertinent to the present illness. A tendency to bruise and bleed easily, the latter having been noted particularly following dental extractions, had been manifested for many years. Three years prior to admission, a prolonged hemorrhage occurred following a miscarriage. One year later, following delivery of a normal child, there occurred a hemorrhage of sufficient severity to require a transfusion. During this pregnancy, 2½ years prior to admission an enlarged spleen was discovered. It was the patient's opinion that the mass had not increased in size since that time. The patient had always been of tense, anxious disposition and had not changed in this respect over a period of ten years. In view of the tendency to bleed and splenomegaly, the patient was referred for study.

Physical examination revealed a small, poorly nourished young woman of

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† Resident

‡ Interne

swarthy complexion There were firm, discrete small lymph nodes palpable in the left axillary and left inguinal regions The skin was warm, moist, with marked palmar and plantar hyperhydrosis, and moderate dermatographia was demonstrable Bilateral pinguiculae were present There was slight bleeding from the gums A massive spleen was felt, which was firm, non-tender, extended to the pelvic brim and occupied almost the entire left abdomen The physical examination was otherwise not remarkable

Laboratory findings of significance included normal urinalysis and stool examination, doubtful serologic test for syphilis,<sup>1</sup> normal RBC (4.71 million), with hemoglobin of 13.5 Gm, hematocrit 41.8 (MCV—89, MCH—29, MCC—32), normal WBC (6,500), with Juvenile neutrophils 7.0%, Segmented neutrophils 65.0%, Eosinophils 1.0%, Atypical lymphocytes 0.5%, lymphocytes 21.5%, monocytes 5.0%, normal osmotic fragility test, platelet count by the Rees-Ecker method 62,000, markedly positive tourniquet test with showers of petechiae appearing after five minutes of compression with pressure midway between systolic and diastolic tensions (115 mm of Mercury), Bilirubin 1.6 mgm per 100 cc of serum of which less than 0.8 mgm reacted directly, cholesterol 110 mgm per 100 cc of serum, cephalin flocculation 3+, thymol turbidity 11.2 units

In view of the splenomegaly and thrombocytopenia, a sternal puncture was performed, Dr C Lockard Conley interpreted this as follows "There is an abundance of rounded and ovoid cells 30 to 50 MU in diameter, of somewhat indistinct outline, the cytoplasm of which stains poorly, with faint bluish cast and a fine wavy fibrillar structure The nuclei are small and distinct, often eccentrically placed and many cells are multinucleated " These cells were felt to be typical Gaucher's cells The megakaryocytes were numerous, but the platelets were reduced in quantity

After establishment of the diagnosis of Gaucher's disease, it was felt that the thrombocytopenia and megakaryocytic arrest were on the basis of secondary hypersplenism, and in view of the history of bleeding it was deemed advisable to perform a splenectomy On 8/2/47 a splenectomy and liver biopsy were carried out by Dr Alfred Blalock under nitrous oxide, ether, and oxygen anaesthesia, with removal of a spleen weighing 2300 gms Within four hours postoperative, the platelets had increased to 116,000 and by the second postoperative day had reached 241,000 On the eleventh postoperative day the platelets reached 424,000 Concomitant with the rise in platelets, a rise in leucocytes to 19,500 on the second and 14,000 on the eleventh postoperative days and a rise in hematocrit to 45 were observed No significant change in differential count or cell indices was observed Postoperatively the tourniquet test was entirely negative on the third day, the cephalin flocculation fell to 2+, the thymol turbidity to 8.8 units, the cholesterol rose to 181 mgm per 100 cc of serum, and the Bilirubin fell to less than 0.8 mgm per 100 cc of serum on the eleventh day The prothrombin concentrations both pre- and postoperatively ranged between 50 and 75% of normal

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<sup>1</sup> Slide test persistently positive, Wassermann negative It was our opinion that this was a biological false positive

Microscopic examination of the liver biopsy specimen showed normal liver cells but many large Gaucher's cells in the liver capillaries. The spleen was infiltrated with these cells which formed nests and strands throughout the entire splenic pulp so that little remained of either the red or the white pulp, there were only a few generally atrophic Malpighian bodies scattered throughout the spleen (Dr Richard Folis, Jr.)

The postoperative course was entirely uneventful in all other respects and patient was discharged, greatly improved, with the diagnosis of Gaucher's disease and secondary thrombocytopenia.

Twelve months after operation the platelet count remained above preoperative levels, 192,500, the hemoglobin was 70%, the WBC 18,450 with a normal differential count, and there had been no further tendency to bleed or bruise. (Courtesy of Dr W L Miller, Gadsden, Ala.)

#### DISCUSSION

We believe that this case illustrates selective depression of a circulating blood element, in which dramatic remission followed removal of the spleen laden with Gaucher's cells, and that the thrombocytopenia was a manifestation of hypersplenism. Such diseases as congenital hemolytic icterus, idiopathic thrombocytopenic purpura, and primary splenic neutropenia have been considered varieties of hypersplenism. However, the picture of hypersplenism has been reported in association with many other diseases in which splenic involvement occurs, including Hodgkin's disease, tuberculosis, sarcoidosis, Banti's syndrome, etc (1-7).

It is well known that reduction of platelets is a prominent feature of Gaucher's disease, and purpuric manifestations are among the most common physical findings (8-12). In the great majority of such instances the thrombocytopenia is a manifestation of more general reduction of all of the circulating blood elements, with anemia and neutropenia likewise present. In his series of 220 splenectomies, Doan (3) found only two instances of Gaucher's disease, in both of which panhematocytopenia was present, and both were relieved by splenectomy. Other observers have reported similar improvement in the hematologic picture following splenectomy in Gaucher's disease (3-10). That the thrombocytopenia is due to megakaryocytic arrest and not to myelophthisis has been recently emphasized by Dameshek and Miller (6) and confirmed by our case.

The patient herein described demonstrated as the only hematologic abnormality a marked thrombocytopenia with megakaryocytic arrest

The diagnosis was established by the finding of typical Gaucher's cells in the material obtained by sternal puncture, and following splenectomy the platelet count rose to normal levels. This case, therefore, appears to be one of selective hypersplenism secondary to the splenomegaly of Gaucher's disease.

#### SUMMARY

An instance of thrombocytopenia without evidence of neutropenia or anemia on the basis of hypersplenism secondary to Gaucher's disease is reported. Splenectomy was followed by return of the hematologic picture to normal.

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# PROCEEDINGS OF THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

HELD IN HURD HALL, NOVEMBER 8, 1948

*Dr Follis* I should like to open this first meeting of this year of The Johns Hopkins Medical Society

The first speaker on the program this evening is Dr Charles G Zubrod, of the Department of Pharmacology and Experimental Therapeutics and Medicine. The title of Dr Zubrod's paper is "The Need for Intrathecal Therapy in the Treatment of Experimental Meningitis"

*The Necessity of Intrathecal Drug in the Treatment of Experimental Meningitis*  
GORDON ZUBROD (Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University)

An experimental meningitis has been induced in cats by intracisternal injection of *K pneumoniae*. This infection is uniformly fatal in untreated animals. Treatment with intramuscular streptomycin produces a significant number of cures only when relatively large doses are given. Treatment with intrathecal streptomycin or with simultaneous intramuscular and intrathecal drug indicates that cure is a function of the amount of drug in the cerebrospinal fluid. For optimum results in the treatment of this experimental meningitis, one must effect the presence of streptomycin in the cerebrospinal fluid. This can be accomplished certainly by intracisternal administration, and probably by the intramuscular route if large enough doses are given. The clinical implications of these data are discussed.

*Dr Follis* Dr Zubrod's paper is open for discussion.

*Dr Marshall* I have been very much interested in this question since the sulfonamides came out several years ago. Whether it is necessary to give both intrathecal and parenteral administration of therapeutic agents for meningitis has been discussed frequently, but as far as I know, this study of Dr Zubrod's is the first attempt to answer the question experimentally. Personally, I have always thought it was unnecessary to give the drug intrathecally. Apparently, I have been wrong. It appears that drug in spinal fluid alone is effective, whether it gets there by being put in intrathecally or by passing the blood-brain barrier.

I think Dr Zubrod emphasized, and I would like to emphasize again, that with very large doses one obtains as good results with intramuscular doses alone as with the combined dosage. I think this is a very important point and must be kept in mind.

Dr Dowling—I happen to have a copy of his paper, it is not published yet, but is in the press—actually had 10 cases of pneumococcal meningitis that were treated with intramuscular penicillin alone, of these, 9 recovered. They received penicillin

intramuscularly, 12 million units per day, getting it every 2 hours. The very large doses given, allowed a sufficient amount of penicillin to pass over into the spinal fluid to accomplish the result.

*Dr Follis* Are there any other comments on Dr Zubrod's paper?

*Dr Rich* Gentlemen, I was a little baffled—perhaps I understood it wrongly—but I thought Dr Zubrod suggested that you should give large intramuscular doses together with the intraspinal. As I see it from the figures, it seems to me it doesn't matter what you give intramuscularly if you give intrathecal doses.

*Dr Zubrod* The reason I suggested that, Dr Rich, was because in the treatment of clinical meningitis the antibiotics are usually given in the lumbar subarachnoid space. The distribution of drugs from the lumbar region upwards is sometimes erratic and rather limited. If you are using small doses of intrathecal drug in the lumbar area, I think you should also use enormous doses intramuscularly in the hope that the blood-brain barrier will allow passage of the drug into the ventricular and cisternal fluid. The results with the experimental meningitis were accomplished with intracisternal injections, one cannot be sure that the same results could be achieved by the use of the lumbar route.

*Dr Follis* Is there any other discussion of Dr Zubrod's paper?

The second presentation this evening will be by Dr Allan L. Grafflin of the Department of Anatomy, whose title is "The Excretion of Fluorescein by the Liver under Normal and Abnormal Conditions."

*Studies of Fluorescein Excretion by the Liver Under Normal and Abnormal Conditions*

ALLAN L. GRAFFLIN (Department of Anatomy, Johns Hopkins Medical School)

The excretion of fluorescein by the liver, under normal and abnormal conditions, has been investigated in male specimens of *Rana pipiens*, receiving fluorescein (uranin) in standard dosage of 30 mg per kg. The course of excretion was observed in vivo with the fluorescence microscope.

Under normal conditions, after injection of fluorescein the dye appears promptly in the plasma, then in the hepatic cells and the bile canaliculi. The peak of excretion (all bile canaliculi sharply delineated at high intensity, all hepatic cells diffusely stained at low intensity) is reached in approximately one hour at ca. 15 degrees C. The sequence of events from the peak of excretion to complete elimination from the liver is essentially a progressive diminution in fluorescent intensity of the dye to non-detectable levels, achieved first in the hepatic cells, then (much later) in the bile canaliculi. As long as the bile canaliculi are delineated they persist as a continuous system, without detectable interruptions in their course. Elimination is usually complete in 24 hours or less. Localized enlargements of the axial bile canaliculi and dilatations at the distal ends of their intercellular branches are repeatedly observed, and are regarded as normal variants of the bile canalicular picture during the excretion of fluorescein.

The present observations upon the normal mechanism of fluorescein excretion



have failed to confirm the findings of A Hirt, J Ansorge and H Markstahler (Ztschr f Anat u Entwickl, 109, 1, 1939) in the later stages of excretion (extensive sprouting of fluorescein-containing vacuoles from bile canaliculi, widespread intracellular vacuole formation, discontinuity of bile canaliculi, "lakes" of fluorescein) However, the whole range of these findings has been observed in animals subjected to highly abnormal conditions, and the distortion of normal function when the liver is subjected to prolonged observation, in vivo, with the fluorescence microscope has been clearly demonstrated

The author's observations have been reported in full elsewhere (American Journal of Anatomy, volume 81, page 63, 1947)

*Dr Follis* Dr Grafflin's paper is open for discussion

If there is no discussion we will pass on to the third and final paper of this evening's program, which is by Dr Manfred M Mayer of the Department of Bacteriology of the School of Hygiene, who will talk on "Recent Advances in Immunochemistry"

*Recent Advances in Immunochemistry* MANFRED M MAYER (Dept of Bacteriology, School of Hygiene and Public Health)

Precise chemical methods for the quantitative measurement of the precipitin and agglutinin reactions were introduced about 15 to 20 years ago and have since proved invaluable to theoretical studies in immunology as well as to the solution of various problems in medicine and biology

For example, accurate determinations of the ratio of albumin to globulin in serum and spinal fluid have been made by immunological technics, with results corresponding to those obtained by electrophoretic analysis Similarly, it has been shown that type-specific pneumococcal polysaccharide can be used as an indicator substance in determinations of blood volume In studies on plant and animal viruses antibody has been used as a specific reagent for quantitative determination of the degree of purification Thus it could be shown that highly purified tobacco mosaic virus was free from constituents of the host plant, while highly purified influenza virus contained large amounts of host material In studies on anaphylaxis and hypersensitivity application of the quantitative precipitin method has made it possible to secure more strictly comparable and reproducible results Striking advances have also been made in blood group studies

Subsequently, the quantitative precipitin method has been extended to the chemical determination of complement in guinea pig, human and bovine serum These studies proved that complement is an actual substance, namely a protein constituent of serum, rather than a "colloidal state" of fresh serum, as had been claimed not so many years ago

More recently, technics have been developed for the quantitative and objective measurement of the hemolytic activity of complement, and their use has made

possible a considerable improvement in the reliability of complement fixation tests. In the course of these studies it could be shown that  $Mg^{++}$  is an essential co-factor in hemolysis by antibody and complement, a fact of theoretical significance as well as practical utility.

During the past 2 years the work in our laboratory at the School of Hygiene has been concerned mainly with the mechanism of hemolysis by antibody and complement. Studies on the velocity of immune hemolysis have shown that in the presence of excess complement and limited amounts of antibody the kinetics of hemolysis resemble those of enzymatic processes. Assays of hemolytic antibody should therefore be based on velocity measurements rather than end point titrations. Such a technic has now been developed and is being utilized at present in studies on purified antigens from erythrocytes and on the nature of the various antibodies present in anti-erythrocyte sera. It is hoped that in the future similar technics can be used in the immunochemical analysis of antireticular cytotoxic serum which has been claimed to be beneficial in wound healing.

In general, immunochemical investigations in recent years have been directed toward the use of highly purified antigens and the quantitative chemical measurement of their interaction with antibody.

*Dr Pollis:* Dr Mayer's paper is open for discussion.

*Dr Turner:* Mr Chairman, Immunology is frequently spoken of as the distinguished offspring of bacteriology. The two touchstones of immunology are the antigen, on the one hand, and the antibody on the other. An antigen is ordinarily defined as a substance that provokes the production of an antibody. And what is the definition of an antibody? A substance that reacts to an antigen!

So, however diffuse these definitions might be, it seems evident that the materials with which the modern immunologist works are far more precise. Dr Mayer has indicated ways in which these methods might be used. I need only to point out that there are many practical applications—among others, in the further identification, purification and isolation of antigens used in vaccines. The typhoid bacillus, for example, is a most complex organism containing numerous antigens—flagella, somatic and the so-called V I antigens—yet despite this knowledge we simply grow the organism in bulk, add a little phenol or formalin, and inject this into human beings in the hope that we will get some immunity. I think it is obvious that with modern immunochemical methods a great deal of work can be done toward developing more potent vaccines, not only for typhoid, but for various other diseases.

*Dr Pollis:* Is there any other discussion of Dr Mayer's paper?

*Dr Vandegrift:* How do you calibrate your antigen? How do you stabilize your antibody?

*Dr Mayer:* You mean the hemolytic antibody? We usually maintain hemolytic sera under aseptic conditions and, if possible, we keep the sera frozen. Under these conditions they are stable. That may not be true for all antibodies, but

certainly for those we work with We check occasionally and we rarely run into difficulty, provided serum is not contaminated by micro-organisms Does that answer your question?

*Dr Vandegrift* Not entirely How do you freeze them? At what temperature are they maintained

*Dr Mayer* We put them in the deep freeze at a temperature of about  $-20^{\circ}\text{C}$  We just like that because that means we can go back into a bottle occasionally without having to take extensive aseptic precautions, but I have also kept many sera in the icebox in the liquid state without loss of activity We have made analyses of the weight of precipitable antibody, even years apart, and never found very much of a difference I can't recall a single instance where a serum analyzed by the quantitative method has seriously deteriorated Of course, I might add that we usually work with sera from hyper-immunized rabbits, and what I am saying need not be true for convalescent sera containing very low levels of antibody

*Dr Follis* Is there any other discussion of Dr Mayer's paper?

*Dr Rich* I would like to ask whether it is conceivable that the antibody is not acting as if it were an enzyme but is acting on the cell and the lysis of a cell releases it

*Dr Mayer* Well, I'll tell you, Dr Rich, we have looked for that and I didn't mention the experiments tonight, but we have made experiments that show such an effect What we did, essentially, was to lyse a suspension of red cells completely with antibody and complement and then collect the stromata, i e the insoluble structural parts of the red cell, by centrifugation These were then washed very extensively—six, seven or eight times—until there was not the slightest trace of antibody in the washings, and then these stromata to which the bulk of the antibody is still bound were suspended in saline and mixed with a portion of red cells No hemolytic antibody was added On incubation nothing would happen However, on addition of complement the new portion of cells lysed

Now, that doesn't prove conclusively that antibody combines with a red cell, does its work, comes off again and goes on to another red cell, because it is just possible—in view of the fact that antibodies are presumably multivalent, that an antibody molecule attached to a stroma particle with one of its reactive groups can react with a red cell by means of another reactive group, and will thus accomplish lysis of the new red cell That is a proposition that can be checked experimentally and it is just the pressure of other experiments that has kept me from investigating it I think one can readily follow that microscopically

I might add, of course, that I feel quite strongly that the experiments described do not prove that antibody acts like an enzyme, but they suggest that there is a similarity in behavior of hemolytic antibody and enzymes

*Dr Rich* Of course, I meant it in a little more crude fashion than that I'd like to know if it really does enter in the combination, erupt mechanically or is shaken loose

*Dr Mayer* Well, one can show very readily that the bulk of the antibody that

you put into a red cell suspension with complement is still attached to the stromata after lysis. It is necessary to subject the suspension of stroma to special treatment in order to dissociate antibody. The results we obtained are probably due to the fact that a very small amount of antibody is in the fluid phase in equilibrium with antibody in combination. When red cells are added to the stromata combined with antibody, the equilibrium is displaced and antibody transfers from the stromata to the red cells.

*Dr Follis* Is there any further discussion?

The meeting is adjourned

## BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal)

*Handbook of Communicable Diseases, 2nd edition* By FRANKLIN H TOP 992 pp Illus The C V Mosby Company, St Louis \$9 50

In the composition of the 1947 edition of his *Handbook of Communicable Diseases* Dr Top has encountered the difficulties of keeping abreast of the rapidly changing fields of new chemotherapeutic, antibiotic, and antihistaminic agents. No sooner is a book such as this on sale, and perhaps before its final proofreading than there are major changes that might well be made. Such situations may be considered as beyond the author's control. This new edition is a distinct improvement over its 1941 parent. The addition of new chapters such as those by T J Abernathy on Primary Atypical Pneumonia and T Francis Jr on Infectious Hepatitis are worthwhile as concise articles on their respective subjects. The chapter on Tuberculosis, though short, is commendable in its clarity. The section on Poliomyelitis is one of the book's best, presenting an interesting and brief discussion of physiotherapy based on the Sister Kenny Treatment. Dr Top's obvious wealth of experience in Communicable Diseases binds the various chapters together and makes for good continuity. The photographs in colors and black and white from the Herman Kiefer Hospital in Detroit, and through the courtesy of the Parke, Davis and Company are excellent. The book reads easily and rapidly. Ample space is devoted to the details of nursing care.

There are still many instances wherein this volume is lacking. The chapter on Diphtheria does not mention the use of antihistaminics in the treatment of serum sickness following serum therapy. It neglects completely the use of the EKG in the early diagnosis and follow-up of diphtheritic myocarditis. It recommends the use of laryngeal intubation in preference to tracheotomy in the treatment of laryngeal diphtheria, the former procedure is considered by many others to be much more difficult to perform satisfactorily, and more fraught with dangers of complicating laryngeal stenosis. The section on Syphilis is perhaps justified in its consideration of penicillin therapy as experimental and without standardization at the time of its writing in January 1947. However, the continued recommendation that mercurial inunctions be used "in the accepted manner" in treating syphilis in late pregnancy and syphilis in infants is archaic. The appendix, an able compilation of interesting statistics on communicable diseases admitted to the Herman Kiefer Hospital during the years 1927 to 1936, would be far more valuable if comparative studies through the year 1946 were added.

Some of the chapters contain sufficient detail and depth to be of definite value to physician or medical student. Many portions are far too superficial. The

Glossary, containing definitions of such words as "adenitis", "bacteremia", "cachexia", and "debilitated", qualify the book, as does the preface to the first edition, as a text or "handy reference for all such persons whose professional duties necessitate contact with certain communicable diseases or infestations" It cannot be recommended as a student text or as a reference for physicians

M G

*Clinical Laboratory Methods and Diagnosis, Fourth Edition* By R B H GRADWOHL The C V Mosby Co, St Louis Missouri, 1948 Illus 4148 pp \$40 00

This massive work in 3 volumes attempts to present descriptions of most of the laboratory tests and procedures available to the physician, as well as discussion of the clinical situation in which the procedures are employed The extensive increase in the use of the laboratory in every branch of medicine has made it impossible for the clinical pathologist to keep abreast of developments in all fields These volumes provide an example of that failure In attempting to cover so much routine, individual subjects are not only superficially, but inaccurately treated Tests are described without critical evaluation of their specificity or clinical value The material for discussion on various diseases states is often poorly chosen This text will not satisfy the physician or medical student Its only value would appear to be as a reference book for the laboratory technician

C L C

*Vol III* This volume is worthy of separate review, because it contains so much new material and might well be considered as a separate text on Tropical Medicine or Clinical Parasitology Dr Pedro Kouri, who is Professor of Parasitology and Tropical Medicine at the Havana University Faculty of Medicine, Cuba has furnished a great number of excellent photographs These are good and worthwhile and many of them are better than the usual ones presented in standard works on the subject

There is a great need for a really satisfactory textbook on Tropical Medicine, one which might combine the basic parasitological knowledge of a biologist with that of a keen clinician, who has practiced for a number of years in tropical climes This book does not fulfill these desiderata Indeed there are a number of either great exaggerations of clinical pictures or gross errors The description of the symptomatology of the "carrier" of amoeba cysts includes almost every possible neurasthenic symptom that man may have in the tropics The statement that *Endamoeba histolytica* has been recovered from duodenal washings is startling The description of malaria is not only weak, but parts of it are contradictory and to the best of modern knowledge wrong Sporozoites from the mosquito almost surely do not penetrate the red cells of man directly as indicated in the chart on pages 214 and 215 This pictorial representation which is based on Schaudinn's original description has not been confirmed, and there is a wealth of data showing that the parasite is not present in the blood from a short time after infection until

almost the end of the incubation period In the discussion of enterobiasis on page 315 there is no indication of the great superiority of anal swabs of either cellophane or scotch tape when searching for eggs One finds this in small print on page 53, contradicting the main text

This, then, is a book which with a lot of work could be made into an excellent text

F B B

*Viral and Rickettsial Infections of Man* Edited by THOMAS M RIVERS, M D ,  
Director of the Hospital of the Rockefeller Institute for Medical Research  
77 illustrations, including 6 plates in color J B Lippincott Company,  
Philadelphia-London-Montreal, publishers 587 pages \$5 00

*Viral and Rickettsial Infections of Man*, edited by T M Rivers, Director of the Hospital of the Rockefeller Institute for Medical Research and authored by a variety of experts at the Institute or elsewhere, is intended primarily for medical students and practicing physicians As such, it fulfills very adequately a tremendous need for a simplified up-to-date textbook on these diseases The chapters are well written and the information in each case is authoritative Besides the chapters on a variety of different infections of man, there are included short chapters on physical and chemical procedures by Stanley and Lauffer, on serologic reactions by J E Smadel, chick embryo techniques by Goodpasture and Buddingh, propagation in tissue cultures by Enders, epidemiology by Maxcy and bacterial viruses by Hershey and Bronfenbrenner

It is true that most of these particular chapters are of little practical use to the practicing physician in the diagnosis and treatment of the common virus diseases, but basic information is important in these diseases Other chapters which cover specific diseases are of great practical and theoretic interest Particularly welcome is the chapter on trachoma and inclusion conjunctivitis by Phillips Thygeson, for little of this information has gotten into the general literature The chapter on dengue by Albert Sabin also is full of new and hitherto unpublished information It is to be hoped that having written this chapter Dr Sabin will be stimulated to publish the basic information on which it is founded Otherwise workers in virus diseases will continue to have to accept the conclusions on faith

In summary this is an excellent elementary text, which makes no attempt at covering all of the basic information on virus diseases in general, but is of real value in giving general concepts and summarizing knowledge

F B B

*Bacterial and Mycotic Infections of Man* Edited by RENÉ J DUBOS, PH D , The  
Rockefeller Institute for Medical Research 101 illustrations, including 3  
plates in color J B Lippincott Co , Philadelphia-London-Montreal,  
publishers 785 pages \$5 00

This is intended as a companion text to *Viral and Rickettsial Infections of Man* It has been edited and written with much the same point of view The need for

this book is not quite as great as that of the first, for there are a number of fairly adequate recent texts of bacteriology. However, most of the chapters in this book seem to be written with a slightly different viewpoint from the average textbook. The chapters on Parasitism and Disease by Francis, and Epidemiology by Maxcy give a clue to this, for particularly here is recognition given to the influence of Theobald Smith's concepts of the biological nature of disease. The individual chapters by individual authorities again present a high standard of excellence and range, from the general subjects to specific infections. Properties of Bacteria which Enable Them to Cause Disease by MacLeod and Pappenheimer, and the Allergic State by Merrill Chase deserve particular notice.

This book has not been written by clinicians and thus those who search in this text for up to date information on the treatment of some of the bacterial diseases will occasionally be disappointed. For instance, the statement on meningococcal meningitis "Penicillin may be as satisfactory as sulfadiazine, systemic administration of the drug should be supplemented by intrathecal route" (p 514), does not adequately reflect the general use of combined sulfadiazine and penicillin for this infection. The table on p 681 on the "Choice of Chemotherapeutic Agent" lists granuloma inguinale as if it were a virus disease and gives no indication of the established value of streptomycin in this bacterial disease. This is within a chapter which is excellent in general concepts and principles, but such minutiae are important when dealing with particular patients.

In summary this book, like its companion, is a real contribution and an excellent text, but should not be viewed as a clinical text, which it does not really pretend to be. The greatly reduced cost of these two texts is partly due to support from the National Foundation of Infantile Paralysis.

F B B

*Fundamentals of Psychiatry, Fourth Edition* 1948 By EDWARD A. STRECKER  
J B Lippincott Company 303 pages \$4.00

The fourth edition of this standard compendium of psychiatry, revised and amplified, remains a very adequate presentation of descriptive psychiatry vitalized by simplified dynamic concepts. The simplifications of theory and case illustrations may be misleading. There is a strong tendency to cite possible organic causes of mental illness and to stress organic reaction patterns rather than enter into the complexities of personality development and its deviations. A brief new chapter on psychosomatic medicine deals with physiologic dysfunction due to emotional disorders rather than with medical illnesses in which emotional difficulties play an etiologic role. The book retains the easy but ineffectual means of disposing of various asocial and antisocial personalities by labeling them constitutional and lumping them with mental deficiency as defect states. Although the book has many merits as a brief review of well accepted concepts, it cannot be considered a reflection of current thinking concerning the dynamic approach to the understanding of personality function and its disorders.

T L



*Handbook of Orthopaedic Surgery, 3rd edition* By ALFRED R. SHANDS, JR., in collaboration with RICHARD B. RANEY Illus 574 pp \$6 00 C V Mosby Co, St Louis, Mo

This book was very interesting to me. I have read small portions of the first and second editions from time to time and found them useful. The third edition, although it follows the pattern of the first two editions is more concise and complete. This is an excellent book for reference experience if one must prepare lectures to medical students or nurses. The bibliography is especially valuable in this regard. The numerous illustrations bring out the facts forcefully.

I heartily recommend the third edition to all who are interested in the diseases and disturbances of the musculo-skeletal system.

W R F

*Edinburgh Post-Graduate Lectures in Medicine* Volume IV, 1944-1947 Edin burgh, Oliver and Boyd 582 pp 18s net

This volume contains forty-one lectures on diverse unrelated subjects. The quality of the lectures varies tremendously. Much of the material can be better obtained from original sources or other texts. Some of the lectures suffer from a pontifical style which is likely to develop in presentations prepared for delivery.

The varied quality and interest in the articles should not detract from some which are excellent reviews and comments in their field. The volume is valuable only as a library collection of articles which have already been published separately.

E N

*The Thyroid and Its Diseases, 2d ed* By J H MEANS Illus 571 pp \$12 00 J B Lippincott Co, Philadelphia, 1948

The second edition of this popular text is a very welcome help in a field in which there has been tremendous activity and progress in the last ten years. The anti thyroid drugs and radio-active iodine have been responsible for new therapeutic and investigative attack on the thyroid diseases. To write a text in the midst of such rapid developments requires nice judgement and selection of facts. The book's greatest value continues as—"an account of the personal experiences of a considerable group of workers." Every physician should have the book.

E N

*Stethoscopic Heart Records* Columbia Masterworks Set M-735 \$15 00

These records played on a high fidelity phonograph give excellent results. The results are greatly dependent on the qualities of the amplifier and particularly the speaker in the phonograph machine used. A single large speaker will lose much of the high pitched sounds and accentuate or even rattle the low sounds. A small single speaker will better reproduce the higher pitched sounds (as well as the needle scratches and extraneous noises). Thus it is not easy to bring out all that is in these reproductions. They are particularly valuable for training in timing of

sounds, and with a good phonograph a student can learn much about the characteristics of the sounds. They can be studied and repeated often at leisure—the greatest advantage of records

E N

*Treatment of Heart Disease* By William A. Brams. Illus. 195 pp. \$3.50  
W. B. Saunders Company, Philadelphia, Pa. 1948

In the preface Dr. Brams states that he intends this book to be "a systematic and practical guide in the treatment of heart disease" intended primarily for the general practitioner and the medical student. It succeeds admirably in this purpose for the practitioner but falls somewhat short for the medical student.

The author begins with a brief but sound discussion of the pharmacological action of drugs used in the treatment of heart disease, then discusses the treatment of congestive failure in general. Each major etiological group of heart disease is then discussed, again purely from the therapeutic standpoint, the author assuming in each case that the proper diagnosis has been made. He ends with the principles of management of cardiac patients during pregnancy and surgery.

The book is well organized, fairly comprehensive and very clearly written. Dr. Brams speaks with authority based on experience and familiarity with the literature. He admits that the opinions expressed are contested by some cardiologists, but actually there is very little argument to be found with the fundamental principles he expresses. Various cardiologists would make changes in his regimen of treatment of some diseases, but these differences would be based largely on individual preference for the use of certain drugs rather than on disbelief in Dr. Brams's regimen.

The index is well organized and adequate. The bibliography is organized numerically as the references are made in the text, hence serves no purpose as a source of information in itself.

For the practitioner who is interested in a brief, practical guide to treatment of cardiac disorders with a minimum of discussion of alternative regimens, this book will be found most useful. Likewise the medical student who is interested in a summary of commonly accepted and sound regimens for handling cardiac problems will find this book very helpful, but because of its lack of discussion of controversial points, its lack of presentation of different views on these points, and its meager reference to the literature, it should not be recommended to medical students as their sole text on the treatment of heart disease.

R M

*Treatment by Diet, 5th edition* By CLIFFORD J. BARBORKA, M. D. Philadelphia  
J. B. Lippincott Co. 1948. Cloth. Pp. 784. Price \$10.00

This book of 784 pages attests to the continued need for better and more widespread information on "Treatment by Diet." This new fifth addition shows the author's same conscientious attempt to simplify the subject as he demonstrated in preceding editions. It reveals an awareness, as the author states that "Intelli-

gent treatment by diet is the greatest weapon available to preventive medicine" In view of this declared concept one is surprised that the book is not as practical, as useful, as concise, as clear and as firmly founded on sound physiology and nutrition as the author undoubtedly intended it to be In spots the text is ambiguous For example on page 97 he states "When protamine zinc insulin is used alone it is often advisable to give about a 100 gram serving of 10 or 15% fruit at bed time" On page 98 he continues "When the morning administration of protamine zinc insulin is first commenced, it may sometimes be difficult to adjust the dose and the diet so as to avoid early morning hypoglycoemia A light meal of a glass of milk or a cup of tea with soda biscuits shortly before bed time has been efficacious" Such statements are, to say the least, confusing No where in the text is there mention of the desirability of the bed time meal being made up of food which will supply available glucose several hours after ingestion rather than immediately

The author is to be commended upon keeping the protein in his high calorie diet lists well within normal limits However he neglects to mention the importance of a high protein intake in cases of emaciation due to febrile disorders, etc It seems lamentable that only one page is devoted to the subject of diet in pregnancy and lactation and that the advice given as to the modification of the normal diet in these two conditions is identical It is obvious that the author has not correlated the Recommended Dietary Allowances of the National Research Council for pregnancy and lactation which he reprints on page 710 with the diet he recommends His suggested addition of "one pint of milk" to the diet in health outlined on pages 58-59 fails to take care of the increased need of ascorbic acid in either condition or the demand for calcium and protein in lactation The careful reader will observe that on page 604 the author discusses protein needs in pregnancy and lactation but he fails to mention on page 613 the advisability of referring to these comments so they will quite likely be lost to those using the book as a quick reference One can not fail to wonder as to the selectivity employed in this book when less than one page is devoted to the discussion of diet in the omni-present conditions, pregnancy and lactation, while forty pages are given over to the less frequently used ketogenic diet

In the treatment of pernicious anemia the author recommends liver in abundance "up to 500 grams a day" The efficacy of such therapy is unquestioned but that it has given way to more practical, less expensive and more acceptable therapy is widely recognized There are ideas sprinkled throughout the book which seem out-moded It is to be hoped that the sixth edition will be brought up to date which will enhance its usefulness

E R T

*Principles Governing Eye Operating Room Procedures* By EMMA I CLEVENGER

Illus 215 pp \$5 50 The C V Mosby Co, St Louis, Missouri

Miss Clevenger's complete and careful presentation of operating room technique for nurses will be of great assistance as a guide to new departments in ophthal-

mology as well as a reference for established departments. The importance of the many small details of operating room management is emphasized as the basis of successful nursing support.

The excellent illustrations of the instrument tray set ups for the different procedures are especially valuable.

The manual will definitely take its place as an excellent reference book.

F. W.

## BOOKS RECEIVED FOR REVIEW

- Blood Clotting and Allied Problems* Transactions of the First Conference, Feb 16-17, 1948 Publication of the *Josiah Macy, Jr Foundation*, New York, N Y pp 179
- Conditioned Reflexes and Neuron Organization* By Jerzy Konorski Illus 267 pp \$4 00, published Nov 9, 1948 by *Cambridge University Press, The Macmillan Co* New York, N Y
- Diabetic Manual* By Elliott P Joslin Published by *Lea and Febiger*, 260 pp illus 1 plate in color \$2 50 (Oct 1948)
- Human Biochemistry* By Israel S Kleiner Published by *C V Mosby Co* 1948, pp 649 \$7 00
- Intern's Manual* Published by *W B Saunders*, 201 pp \$2 25
- Medical Statistics from Graunt to Farr* By Major Greenwood, *Cambridge University Press*, Nov 9, 1948 \$2 00
- Pathology* By W A D Anderson Published by *C V Mosby Co* 1948 1453 pp \$15 00
- Experimental Immunochemistry* By Elvin Kabat and Manfred Mayer, 567 pp \$8 75 Published by *Charles C Thomas*, Springfield, Illinois
- Industrial Hygiene and Toxicology* (in two volumes) Vol 1, by Frank A Patty, pp 531 \$10 00 Published by *Interscience Publishers, Inc* 215 Fourth Ave New York, N Y
- Report of the Sanitary Commission of Massachusetts 1850* By Lemuel Shattuck and others Published by the *Harvard University Press*, Cambridge, Massachusetts, 321 pp \$4 50
- Biological Reactions Caused by Electric Currents and by X-rays* By J Th van der Werff, 203 pp \$5 00 Published by *Elsevier Publishing Co, Inc* 215 Fourth Ave New York 3, N Y
- Cancer of the Esophagus and Gastric Cardia* By George T Pack, published by *C V Mosby Co* St Louis, Missouri, 192 pp \$5 00
- Factors Regulating Blood Pressure* Transactions of the Second Conference, Jan 8-9, 1948, pp 170 Publication of the *Josiah Macy, Jr Foundation*, N Y
- The Basis of Chemotherapy* By Thomas S Work and Elizabeth Work, pp 465 \$6 50 Published by *Interscience Publishers Inc*, New York
- The Renal Origin of Hypertension* By Harry Goldblatt Published by *Charles C Thomas*, Springfield, Ill 126 pp \$2 75
- The Selected Writings of William Clowes* By F N L Poynter, pp 179, 15 s Published by *Harvey and Blythe Lmtd*, London, W I

# TISSUE CULTURE STUDIES ON LIVER CELLS OF ANAPHYLACTICALLY (ARTHUS) SENSITIZED ANIMALS IN THE PRESENCE OF THE SENSITIZING ANTIGEN

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A difference between the anaphylactic type of hypersensitivity and the tuberculin type has been clearly demonstrated in tissue cultures of fibroblasts, polymorphonuclear leucocytes, lymphocytes, macrophages, and young marrow cells, by Rich and Lewis (1) and Aronson (2, 3). This difference consists in the fact that the washed cells of these types from an animal showing the tuberculin type of hypersensitivity are injured and killed by contact with the specific antigen in tissue cultures, whereas cells of these types from an animal sensitized anaphylactically to the degree of reacting with a necrotizing Arthus phenomenon are not visibly affected by *in vitro* contact with the specific antigen. These results are unaffected by the presence or absence of antibody in the culture medium. Furthermore, Rich and Folis (4) showed that to elicit the Arthus phenomenon in the cornea of a rabbit the cornea first has to be made vascular. They came to the conclusion that in the anaphylactic Arthus phenomenon there is vascular sensitivity, but the cells of the tissues at large are not sensitized, and that the tissue death in the Arthus reaction results primarily from impairment of nutrition by vascular damage.

It is known that focal necroses of the liver may occur when the specific antigen is injected into the circulation of the anaphylactically sensitized animal under conditions which permit the survival of the animal. Longcope (5) suggested that these focal necroses of liver cells might be due to a toxic substance liberated either into the blood stream, or inside the liver cells, during the anaphylactic reaction. Aptiz (6) thought that the necroses might be due to passive congestion, but since they are often periportal rather than central there would seem to be little support for that view. Hartley and Lushbaugh (7), in more recent studies, concluded that the focal necrosis that occurs

under these conditions is due to contact of the antigen with antibody in or on the liver cells and is not dependent upon vascular damage. Since the question of the sensitivity of epithelial cells of the anaphylactically sensitized body has not been previously examined by *in vitro* studies (which are the only means suitable for determining whether the injurious effects of the specific antigen are due to a direct action upon the cells in question), the present *in vitro* study of the effect of antigen upon the liver cells of anaphylactically sensitized animals was conducted.

#### EXPERIMENTAL METHODS

Adult male albino rabbits, weighing about 2500 gms, were used. They were kept in separate cages and were supplied daily with an abundance of Purina Chow and water.

Sterile horse serum, without preservative, was used as the antigen. The serum was injected subcutaneously into the rabbit for several weeks, in amounts of 1 cc every three days. Skin tests for hypersensitivity were made at intervals by the injection of 0.1 cc of horse serum into the skin of the flank. Sensitization was continued until the test injection produced an Arthus phenomenon with necrosis. Ten days after this degree of hypersensitivity had been attained, a piece of liver was removed by biopsy under aseptic precautions, using ether anaesthesia. This biopsy was used for tissue culture. Immediately after the operation the skin test was repeated. Only when necrosis recurred on this re-test were the results of the culture of the liver tissue considered significant. For every experiment in which liver from an Arthus sensitized rabbit was cultured, a control experiment was carried out, in which liver from a normal rabbit was used.

#### TISSUE-CULTURE METHODS

Blood was obtained from the normal and the hypersensitive rabbits aseptically by cardiac puncture. Graded amounts of Lederle's synthetic heparin were added to 10 cc portions of blood, in order to obtain satisfactory plasma for use in cultures. A dilution of 1-50,000 proved most satisfactory. Serum was obtained from the remainder of the blood.

The media used were made up in two parts. The first (Part 1) con-

sisted of one part beef embryo extract, one part balanced salt solution, Gey (8), and four parts of serum, rabbit serum alone or with horse serum in proportions given in Table I. The second (Part 2) consisted of one part chicken plasma and three parts rabbit plasma. In each experiment a piece of liver removed at biopsy was minced, under aseptic precautions, with detachable knife blades<sup>1</sup> into pieces approximately 0.5–1.0 mm in diameter. Four of these small pieces of liver were

TABLE I  
*Outline of typical experiment*

NO. CULTURES	WASHED LIVER CELLS FROM	PLASMA + SERUM FROM	PERCENT OF HORSE SERUM IN MEDIUM	NO. OF CULTURES SHOWING GOOD GROWTH AND VIABILITY OF LIVER CELLS
35	Arthus Rabbit	Arthus Rabbit	0	30
35	" "	" "	30%	31
35	" "	" "	10%	23
35	" "	Normal Rabbit	0	21
35	Normal Rabbit	Arthus Rabbit	0	31
35	" "	" "	30%	30
35	" "	" "	10%	28
35	" "	Normal Rabbit	0	19
10	Arthus Rabbit	Arthus Rabbit	1:100–100%	8
10	" "	" "	1:1,000	9
10	" "	" "	1:10,000	8
10	Normal Rabbit	" "	1:1,000	8

placed on a sterile cover slip (30 × 35 mm), and three drops of Part 1 were added, followed by two drops of Part 2. Clotting resulted promptly after the addition of Part 2. The cover slip was then inverted and sealed with vaseline to a metal ring attached to a slide with vaseline. The cultures were then placed in an incubator, maintained at 37°C, and were examined daily. Generally at the end of four to six days the cultures were fixed in Vandergrift's solution (9) for five minutes. They were then allowed to stand overnight in 1% ammonia alcohol (95%), then they were washed, stained in dilute Giemsa (20 drops to 50 cc of water) for 24 hours, differentiated in 95% alcohol and dehydrated in acetone and xylene.

<sup>1</sup> Bard Parker, size 11



In five experiments four types of media were used, in order that the cells of the sensitized and of the control animals could be studied in the presence or absence of the specific antigen, and in the presence or absence of antibody-containing rabbit plasma. One hundred and forty cultures, each containing four explants per culture, were made from the liver of each animal. Thus there were 560 explants of the liver of each animal in each of these experiments. In one experiment in which greater dilutions of the antigen were used only 40 cultures were made (Table I).

### RESULTS

The growth of liver cells extended in sheets into the zone around the explants on about the third day. These cells were large, with abundant, pale staining cytoplasm, the margins of which were quite sharply outlined. Their nuclei were large, oval or round, rather vesicular, had a very sharply defined limiting membrane and generally contained one large nucleolus. The cells as well as the nuclei varied considerably in size, some being two to four times larger than others. To differentiate the growth of bile duct epithelium from that of liver cells, several cultures of the epithelium of the common bile duct were made. The latter cells were different in appearance from the liver cells. They grew out in sheets, but the individual cells were smaller and more regular in size, and their nuclei were also much smaller than those of the liver cells (Figures 1 and 2).

Microscopical study of the growing and stained cultures did not show any qualitative or quantitative difference between the growth or appearance or duration of viability of the liver cells of normal rabbits growing in the presence of horse serum, and those of the hypersensitive rabbits growing in the presence of horse serum, whether in the presence or absence of antibody. If anything, both the normal and the hypersensitive cells grew somewhat better in the culture medium that contained horse serum than in the medium without horse serum (Figures 3 and 4).

### DISCUSSION

In the present experiment, animals were sensitized anaphylactically to the degree that they responded with a necrotizing Arthus phenom-

enon to the intracutaneous injection of 0.1 cc of the antigen, at which time their liver cells were exposed to contact with the specific antigen in tissue cultures. The results show that, even in so highly sensitized an animal, the epithelial liver cells, as has been previously shown for macrophages, polymorphonuclear leucocytes, lymphocytes, fibroblasts and young marrow cells, are not damaged *in vitro* by direct contact with the specific antigen. This is in marked contrast to the state of affairs in tuberculin type hypersensitivity, in which, as shown by Rich and Lewis (1) and others, the cells so far tested (polymorphonuclear leucocytes, lymphocytes, fibroblasts and immature marrow cells) are killed by contact with the specific antigen *in vitro*. Indeed, Moen (10) has shown that the cells from animals with tuberculin type hypersensitivity retained their hypersensitivity for numerous generations in tissue culture.

The liver cells from the sensitized animals in the present experiments were exposed to concentrations of the specific antigen ranging between 1-3 and 1-10,000, in the presence and in the absence of specific antibody. In no case was any specific inhibition of growth or viability detectable. The present experiments, therefore, offer no support for the view, suggested by others, that the epithelial hepatic cells of the Arthus sensitized body are, themselves, sensitized in a manner that would cause them to be injured by direct contact with the specific antigen. The epithelial hepatic cells of the Arthus sensitized body thus correspond with the fibroblasts, macrophages, polymorphonuclear leucocytes, lymphocytes, and immature marrow cells in being unaffected by contact with the specific antigen *in vitro*. This would suggest that the focal necroses that occur in the liver of the Arthus sensitized animal, following the intravascular injection of the antigen, result from a vascular alteration, just as the available evidence, reviewed above, indicates that the necrosis of connective tissue at the site of an Arthus reaction is due, not to a direct action of the antigen upon the connective tissue cells, but to interference with the local blood supply. The significant vascular alteration responsible for the focal death of liver cells during protracted anaphylactic reactions may be only an alteration of permeability, which, in itself, could lead to impairment of the nutrition of the liver cells, or there also may occur focal ischaemia, resulting from focal vascular spasm, such as that

observed to occur during focal anaphylactic reactions by Abell and Schenck (10)

We wish to acknowledge the generous cooperation and advice of Dr G O Gey

### CONCLUSION

Epithelial hepatic cells from Arthus sensitized animals are unaffected by contact with the specific antigen in tissue culture, whether in the presence or absence of specific antibody. In this, they correspond with the other types of cells of the anaphylactically sensitized body that have been tested so far (connective tissue, macrophages, lymphocytes, polymorphonuclear leucocytes, immature marrow cells)

The focal necroses that occur in the liver of the anaphylactic body when the specific antigen circulates in the sinusoidal blood stream are not, therefore, due to a direct action of the antigen upon the epithelial liver cells, as has been postulated, but are probably due to an effect of the antigen upon the vascular endothelium which, in the anaphylactically sensitized body, is well known to be injured by contact with the specific antigen

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FIG 1 NORMAL LIVER CELLS IN 30% HORSE SERUM  $\times 100$   
 FIG 2 LIVER CELLS FROM A HYPERSENSITIVE ANIMAL IN 30%  
 HORSE SERUM  $\times 100$   
 FIG 3 NORMAL GALL BLADDER EPITHELIUM  $\times 100$

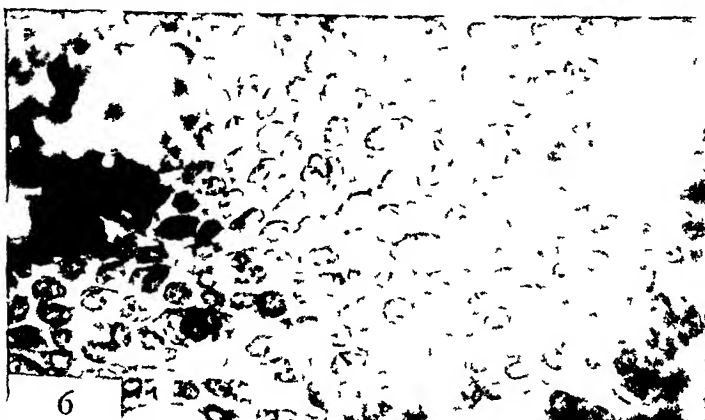


FIG 4 NORMAL LIVER CELLS IN 30% HORSE SERUM  $\times 400$   
 FIG 5 LIVER CELLS FROM A HYPERSENSITIVE ANIMAL IN 30%  
 HORSE SERUM  $\times 400$   
 FIG 6 NORMAL GALL BLADDER EPITHELIUM  $\times 400$

# FAILURE OF GLUCOASCORBIC ACID IN THE DIET TO PRODUCE SCURVY IN MICE

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In 1943, Woolley and Krampitz (1) described in mice and cotton rats a "scurvy-like" condition which was produced by the inclusion of glucoascorbic acid, an analog of ascorbic acid, in the diet. Such animals failed to grow, developed diarrhea and exhibited subcutaneous and gingival hemorrhages. At autopsy "the joints of the legs and ribs were fiery red," and by x-ray examination the bones appeared less dense than those of controls. The addition of ascorbic acid to the diet had no effect on the course of the syndrome, which could be prevented only by the removal of glucoascorbic acid from the ration. Of further interest was the observation that the effects of this material could be produced only on a highly purified diet, not one containing natural food stuffs. Later Woolley (2), using guinea pigs fed a purified diet, found that the addition of ascorbic acid prevented the deleterious manifestations of glucoascorbic acid, and thus concluded that the disease could be "viewed as an ascorbic acid deficiency."

The effects of glucoascorbic acid were further studied by Banerjee and Elvehjem (3) in rats, chicks, and guinea pigs. These investigators confirmed the clinical picture of loss of weight and diarrhea but were unable to find any hemorrhages. The effects of feeding glucoascorbic acid could be prevented by the addition of liver powder to the synthetic diet. Determinations of ascorbic acid concentration in the liver and kidney failed to show any difference with or without the addition of glucoascorbic acid to the diet.

The production of scorbic lesions in an animal such as the mouse or rat would offer a unique chance to study not only changes which are ordinarily not seen in these species but also certain coincident deficiency states, particularly rickets and scurvy which, when they occur together, produce a most confusing morphological picture. It was felt, therefore, that a morphological study of the bones of animals placed on a

glucoascorbic acid containing diet should be carried out to settle the question as to whether or not scurvy could be produced

#### EXPERIMENTAL

The diet which was employed was identical with the highly purified ration described by Woolley and Krampitz (1) Glucoascorbic acid<sup>1</sup> was added to make a concentration of 5%, which the Rockefeller investigators had found adequate to produce the syndrome Ten weanling mice were placed on the glucoascorbic acid added ration, five animals served as controls and ate a similar ration without the above supplement

#### RESULTS

The animals on the glucoascorbic acid added ration grew very little, after a few days most of them lost weight All were dead by the 12th day They were unkempt, their hair was matted by a sticky brownish material In contrast the controls grew well The experimental group developed diarrhea During life and at autopsy no hemorrhages were seen

At autopsy no gross changes could be found Microscopic study of the upper end of the tibia in each animal showed that, beginning at the 3rd day, the cartilage cells appeared to slow their growth activity, the zone of hypertrophic cells becoming narrower During the remaining week, that is, up until the 12th day, the zone of provisional cartilage cells became narrower and narrower, while osteoblastic activity, as evidenced by bone formation at the cartilage-shaft junction, was greatly retarded In other words, the picture produced was a non-specific cessation of both cartilagenous and osteoblastic activity, such as is seen in inanition, and is therefore quite unlike the specific changes which are so pathognomonic of skeletal scurvy (4) There were no microscopic hemorrhages in the bone or beneath the periosteum

#### DISCUSSION

The disease scurvy, which occurs as a result of the deprivation of ascorbic acid in certain species, is characterized by a failure of connec-

<sup>1</sup> Furnished through the kindness of Dr Philip B Gray of the Wallerstein Laboratories, New York 66, New York



tive tissue cells, osteoblasts and odontoblasts to deposit their respective intercellular substances collagen, osteoid and dentine (5) When one finds a disturbance in the formation of one or more of these materials, the diagnosis of scurvy is valid Thus it is apparent that the most precise way to recognize the disease is to study the tissues of the suspected subject From the observations reported above, it is apparent that glucoascorbic acid does not produce changes in the bones characteristic of the scorbutic state The cause for the clinical picture is not clear The diarrhea and failure to grow are reminiscent of changes produced when excessive quantities of lactose or galactose are added to the diet (6)

#### SUMMARY

Morphological studies of the bones of young mice which were fed a diet containing 5% glucoascorbic acid failed to reveal any changes characteristic of scurvy, only the effects of acute inanition could be demonstrated

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# RENAL FAILURE IN EMBOLIC GLOMERULONEPHRITIS AS A COMPLICATION OF SUBACUTE BACTERIAL ENDOCARDITIS

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The recognition of subacute bacterial endocarditis may be difficult in its atypical forms. A combination of septic fever, a cardiac lesion, splenomegaly, anemia, and embolic phenomena is practically diagnostic in the typical case. In the case to be reported the clinical picture of subacute bacterial endocarditis was dominated by the manifestations of renal failure which in turn may have been responsible for the absence of the usual septic temperature curve. Postmortem examination of the kidneys revealed the essential renal lesion to be that of embolic glomerulonephritis, a condition rarely associated by itself with impairment of renal function.

J. H., a 60 year old laborer was admitted to the Surgical Service of the Johns Hopkins Hospital on March 12, 1947, complaining of progressive weight loss of six months duration. He had been referred to the attending surgeon with the diagnosis of gastric carcinoma.

The family history revealed that both parents had died of tuberculosis at the ages of 64 and 74. Two sisters had died of tuberculosis at unstated ages.

The past history was non-contributory. There was no history of chorea, rheumatic fever, or other serious illness.

The present illness had started insidiously six to eight months before admission with weight loss. This had been accompanied by slight dyspnea on exertion and ankle edema. The patient had suffered no abdominal distress, pain, nausea, vomiting or dysphagia at any time.

Several months before admission, the patient had noted the development of nocturia, characterized by the passage of small amounts of normal appearing urine. He had no diurnal frequency, but had been told by his doctor that his kidneys were bad. His vision had started to fail at that time and had become increasingly poor by the time of admission. He had lost 14.5 kilograms. The systemic review was otherwise negative.

*Physical examination.* The temperature was 97°, pulse rate, 78/min, and respiratory rate, 18/min. Blood pressure readings on three separate occasions were 90/70, 130/90, and 110/80. He weighed 38.6 kilograms and was 170 centimeters tall.

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The patient appeared emaciated and dehydrated. He was resistant and combative. The skin showed innumerable petechiae over the back, extremities and abdomen. It was loose, flabby, and showed marked loss of subcutaneous tissue. The muscles were atrophic and atonic but not tender. Movement of the spine was considerably restricted. There was no generalized or local lymphadenopathy.

The head showed no bony lesions or tenderness. The eyes were sunken and staring with normal pupillary reactions and normal extraocular movements. Ophthalmoscopic examination showed haziness of the media bilaterally. One observer was able to make out several large scattered haemorrhages in both retinæ and a large white area surrounded by pigment deposits in the lower temporal quadrant of the right eye.

Examination of the neck showed no goiter or tracheal deviation. The chest was markedly emphysematous. The lungs were clear to physical examination. The heart was slightly enlarged to the left by percussion. The rhythm was regular and slow. The apex impulse was diffuse and slightly displaced to the left. No thrill was felt. A loud, coarse systolic murmur was audible at the apex, but no diastolic murmur could be detected. The peripheral vessels were hard and tortuous. Pulsations in the left foot were diminished.

Abdominal examination showed evidence of marked emaciation, but no masses, rigidity or tenderness. The liver and spleen were not enlarged. The genitalia, prostate, and rectum were essentially normal.

There was no clubbing or cyanosis of the fingers or toes. Aside from the mental status neurological examination was normal.

*Laboratory examinations* The findings on admission were as follows: Hemoglobin, 8.8 gms %; Leukocyte count, 15,250; Serological test for syphilis, negative; Urinalysis specific gravity, 1.010, albumin, +, microscopic, many erythrocytes, 3-4 leukocytes per high power field, no casts; Blood chemistry non-protein nitrogen, 14.3 mgm % and 15.4 mgm %, fasting blood sugar, 124 mgm % and 108 mgm %, chlorides, 106.2 Meq/L, CO<sub>2</sub> combining power, 14.9 Meq/L, Bilirubin, less than 0.8 mgm %.

A chest x-ray showed no evidence of metastases. A gastrointestinal series was reported to be negative. The stomach was described as large, ptosed, but within normal limits.

*Urological consultation* The patient was examined by a urological consultant whose clinical impression was marked chronic bilateral pyelonephritis and cicatricial contracture of the vesical orifice with obstructive changes in the bladder.

The intravenous pyelogram showed poor visualization and filling of the upper urinary tract. Cystoscopy showed contraction of the vesicle neck, hypertrophy of the trigone, and trabeculation of the bladder. Retrograde pyelography was done on the right side only and showed flattening and expansion of the minor calices compatible with the shortening and flattening of the pyramids seen in chronic pyelonephritis. There was no evidence of hydronephrosis.

*Hospital course* The patient was continuously afebrile, but showed no signs of improvement. He remained mentally dull, answered questions slowly, and was

usually uncooperative. A phenolsulfonphthalein test could not be performed because the patient voided on the floor. Repeat urine specimens were difficult to obtain. On the fifth hospital day the NPN had risen to 173 mgm %. Repeat chest x-rays three days later showed no evidence of significant lesions in the lungs or abnormality of the heart's shadow.

The patient was transferred to the Medical Service with a presumptive diagnosis of chronic pyelonephritis with uremia.

The blood chemistry showed the following changes on the ninth day after admission to the hospital: Non-protein nitrogen, 202 mgm % and 250 mgm %; Uric acid, 10.7 mgm %; Calcium, 9.1 mgm %; Phosphorus, 8.9 mg %;  $\text{CO}_2$  combining power, 12.4 Meq/L; Chlorides, 109.2 Meq/L.

The situation was considered hopeless unless a cause for the renal failure amenable to therapy could be discovered. Since chronic pyelonephritis did not satisfactorily explain all the clinical manifestations, diagnostic study was continued. Because of the anemia, purpura, and uremia in the absence of hypertension, multiple myeloma was considered. Further laboratory work was carried out with this possibility in view. The findings of the tenth hospital day were: Erythrocyte count (millions), 2.38; Hemoglobin, 6.7 gms %; Volume packed RBC, 20 %; Mean corpuscular volume, 84 cu microns ( $N = 87 \pm 5$ ); Mean corpuscular hemoglobin, 28 microgamma ( $N = 29 \pm 2$ ); Mean corpuscular hemoglobin concentration 33% ( $N = 34 \pm 2$ ); Icterus index, 2; Sedimentation rate (Win-trobe), 43 mm /C S R = 0; Leukocyte count, 10,450; Juvenile neutrophils, 11%; Polymorphonuclear leukocytes, 83%; Lymphocytes, 2%; Monocytes, 4%; Platelet count, 152,000; Bleeding time, 30 sec ( $N = 60 - 120$ ); Clotting time, 8 min (normal); Clot retraction begins, 1 hour (normal); Clot retraction complete, 17 hours (normal); Tourniquet test (Rumpel-Leeds), negative; Blood Smear showed slight hypochromia, poikilocytosis, and rare polychromatophilia.

Sternal puncture was unsatisfactory in that chiefly peripheral blood was obtained. No plasma or myeloma cells were seen in the material examined. Total serum proteins were 5.6 Gms %, with 2.8 Gms % albumin and 2.8 Gms % globulin, giving an A/G ratio of 1.0. X-ray of the skull, ribs and shoulder girdles showed no evidences of bony lesions. Urinalysis showed the following: Bence-Jones protein, negative; Albumin, ++; Specific gravity, 1.011; Microscopic, many leukocytes and erythrocytes.

Since subacute bacterial endocarditis was considered a remote possibility despite the lack of fever, a blood culture was taken on the 9th hospital day. The heart murmur was explained clinically as due to calcification of the mitral valve with organic insufficiency. An electrocardiogram on March 20th showed only low voltage in the limb leads.

On the tenth day after admission the patient suddenly developed clinical signs of a cerebro-vascular accident. His neck was stiff and he soon lapsed into coma. In view of his moribund condition and poor prognosis, the patient was not disturbed for lumbar puncture. He died the following day.

On the day that he died, the blood culture was reported to show a growth of "Alpha Streptococcus"

*Autopsy* The autopsy (#20493) was performed approximately three hours after death. Examination of the body revealed an extremely emaciated white man weighing only 36.5 kilograms. The entire body was covered by a petechial rash that involved even the conjunctivae and nail beds. Findings of special importance were

*Heart* The heart was not large and weighed only 270 grams. The tricuspid, pulmonic, and aortic valves were normal. A large, V-shaped defect in the posterior leaflet of the mitral valve extended from its free edge almost to the valve insertion and measured approximately 15 mm in diameter. The edge of this defect was covered by smooth endocardium and had chordae tendinae attached to it. There were no fresh or healed vegetations on the valve around this defect. There were soft, brown, friable vegetations on the entire anterior leaflet of the mitral valve and on the left half of the posterior leaflet. The largest of the vegetations was located on the anterior leaflet and measured approximately 15 mm in diameter. The endocardium of the left auricle above the anterior leaflet of the mitral valve was roughened irregularly, involving an area approximately 20 mm in diameter. There was a nodular vegetation 5 mm in diameter in the central portion of this roughened area.

*Urinary system* The right kidney weighed 110 gm and the left, 130 gm. The subcapsular surfaces were smooth and several petechiae were noted on these surfaces. On section neither the boundaries of the cortex nor its striations were clearly defined. Several submucosal petechiae were present in the renal pelvis and urinary bladder. The calices, renal pelvis, and ureters were not dilated. The ureters, urinary bladder, prostate, and urethra were normal with the exception of the submucosal hemorrhages in the urinary bladder.

*Spleen* The spleen weighed 180 gm. It contained three yellow infarcts, two of them measuring about 6 mm in diameter and the third, 18 mm. Each was surrounded by a red halo about 2-3 mm in width.

*Brain* The lepto-meninges were delicate and pale except over the upper surface of the brain where there were yellowish-brown foci. The blood vessels at the base of the brain were delicate and free of arteriosclerotic changes. There was no evidence of a "pressure cone."

On the cut surface of most of the sections through the brain there were petechial hemorrhages in both the white and gray matter. There were several small but distinct areas of softening in the anterior portion of the left corpus striatum. Deep in the left anterior sulcus there was a vessel that appeared to contain a relatively fresh thrombus. In the cortex beneath this thrombosed vessel there was a round hemorrhagic area, approximately 8 mm in diameter.

*Aorta* The aorta was elastic and had a smooth, ivory-like intima throughout. There was a tiny opening less than 1 mm in diameter in the ductus arteriosus.

*Microscopic studies* *Heart* Section through the infected portion of the mitral valve and the left auricle revealed organizing vegetations covered by fibrin.

Clumps of Gram-positive cocci were visible near the surface of the vegetations. Throughout the myocardium, pancreas, spleen, and brain there were myriads of microscopic infarcts. There were a number of mycotic aneurysms in the small blood vessels of the heart, pancreas, spleen, and brain. No fresh or old Aschoff bodies were seen in the myocardium or in the left auricular endocardium. No evidence of inflammatory changes were seen in a section through the edge of the mitral valve defect.

*Kidneys* Sections through the kidneys showed extensive and typical lesions of embolic glomerulonephritis. Although many of the glomeruli appeared normal, the remainder showed embolic phenomena varying from hyaline and fibrous changes in one or more capillary loops to complete fibrosis of a glomerulus. Other glomeruli contained fibrous and epithelial crescents, erythrocytes were seen within the capsules of others. These distinctly focal lesions contrast with the rather uniform glomerular lesions seen in the diffuse subacute glomerular nephritis associated with Alpha streptococcus endocarditis. Many of the tubules contained granular casts, and there were foci of round cells in the renal parenchyma. No organisms were seen in bacterial stains of the kidneys. Bacteria were stained in the large infarcts of the spleen and in the large thrombosed meningeal vessel described grossly in the left anterior sulcus.

*Postmortem bacteriological studies* From a culture of the vegetation on the mitral valve (which was contaminated in removal) Alpha Streptococcus mitis was isolated, along with several common intestinal contaminants. A direct smear of the vegetation itself revealed numerous Gram-positive cocci in pairs and chains. From the heart's blood, a pure culture of Group B Beta hemolytic streptococcus opportunus was isolated, which probably represented a terminal invader.

*The complete final anatomical diagnosis was* Congenital malformation of the mitral valve. Patent ductus arteriosus. Bacterial endocarditis (Alpha Streptococcus mitis) with vegetations on the mitral valve and wall of the left ventricle. Emboli and minute infarcts in the myocardium, spleen, pancreas, and brain, mycotic aneurysms in the coronary, pancreatic, and cerebral arteries, petechial hemorrhages in the skin, fingernail beds, renal pelvises, submucosa of the urinary bladder, conjunctivae, and retina. Extensive embolic glomerulonephritis. Septic choroiditis. Hyperplasia of bone marrow. Purulent bronchitis and lobular pneumonia. Emaciation. Partial optic atrophy and scars of old choroiditis. Atrophy of testes. Fibrous adhesions over lower lobe of right lung. Calcified nodules in the lungs, hilar lymph nodes, and liver. Polyps of the colon.

There was clearly a congenital malformation of the mitral valve, in view of the fact that the deformed valve was normal histologically, and chordae tendinae were attached to the free edge of the congenital defect. Since there were no rheumatic lesions in the heart, the bacterial endocarditis was evidently associated with a congenitally malformed mitral valve. The susceptibility of malformed valves to endocarditis is well known. Embolic nephritis sufficiently extensive to produce renal failure is rare in subacute bacterial endocarditis. As evidenced by the purpuric cutaneous petechiae and myriads of microscopic infarcts in the myocardium

pancreas, and brain, there were obviously widespread minute emboli throughout the entire body

#### DISCUSSION

Fever is generally considered to be a cardinal sign of subacute bacterial endocarditis but, as several authors have stated (1, 2, 3, 4, 5), it may be absent especially when renal insufficiency develops. An interesting feature of the case just reported was the lack of febrile response during the period of observation.

The lesions of focal embolic nephritis were first described by Lohlen in 1910 (6). Baehr and his group of workers, between 1912 and 1923, published a classic series of papers (7, 8, 9, 10, 11, 12, 13) contributing to our knowledge of this condition. Further studies were made by Gaskell (14) in 1913 and in 1932 by Bell (15). As frequently as this lesion accompanies subacute bacterial endocarditis, it is rarely extensive enough to cause renal failure. Primary renal failure developing during the course of bacterial endocarditis is usually the result of diffuse glomerulonephritis (11, 16, 17). Libman is quoted by Fishberg (17) as saying that in 800 cases of subacute bacterial endocarditis observed he had never seen a case of uremia due to embolic glomerulonephritis.

A review of the records of the Johns Hopkins Hospital from 1925 through 1945 reveals 83 autopsied cases in which there was either a diagnosis of Alpha streptococcus endocarditis or of subacute bacterial endocarditis. In only nine of these autopsied cases was there a terminal rise above 80 mgm % in the blood non-protein nitrogen. Post-mortem examination of the kidneys from these nine cases gave the following picture: Chronic diffuse glomerulonephritis, one case; Acute diffuse glomerulonephritis, one case; Subacute diffuse glomerulonephritis, three cases; Essentially normal kidneys, two cases; Focal embolic nephritis, two cases. The two patients with focal embolic glomerulonephritis did not have the extensive renal lesions noted in the present case, and both of them, together with the two patients having apparently normal kidneys, were in frank heart failure terminally, thus accounting for their final azotemia. Therefore, in none of these nine cases could focal embolic glomerulonephritis be considered a major factor in their terminal azotemia.

That hypertension may be absent in cases of "renal failure" in subacute bacterial endocarditis has been mentioned by Libman (18), although he did not specify the type of renal failure or the type of renal lesion, and he gave no supporting figures for his statement.

The absence of hypertension in cases of extensive subacute glomerulonephritis associated with subacute bacterial endocarditis is a matter of interest, since hypertension is, of course, the rule in subacute glomerulonephritis in the absence of subacute bacterial endocarditis. It is conceivable that the absence of hypertension in such cases might be due to weakening of the myocardium as a result of widespread emboli from the vegetation. In four of the five cases of diffuse glomerulonephritis listed above, hypertension was not observed at any time during the hospital stay of the patient. On the other hand, the one case of chronic diffuse glomerulonephritis did have hypertension when admitted to the ward. Death occurred nine days later with a sustained hypertension and clinical signs of uremia (Non-protein nitrogen before death = 215 mg %).

The three above-mentioned cases with subacute diffuse glomerulonephritis, in which the blood pressure was not elevated, did have many small myocardial infarctions from such embolization, while the case of chronic diffuse glomerulonephritis in which the blood pressure remained elevated had a normal appearing myocardium.

Bell (15) has reported five cases of "uremia" (details not given) due to multiple glomerular embolization in a series of 108 autopsied cases of subacute bacterial endocarditis. Other cases of "uremia" due to embolic glomerulonephritis have been mentioned by Middleton and Burke (19) and Sautereau (20). Hamman and Brown mention a case seen by Bloomfield (21).

At least 16 cases of "uremia" caused by focal embolic glomerulonephritis have been reported previous to the present case.

#### SUMMARY

A case of subacute bacterial endocarditis implanted upon a congenitally deformed mitral valve, with multiple embolic phenomena and uremia due to focal embolic glomerulonephritis, is presented. In this case unusual features were (1) the absence of fever during the period of observation, (2) the complete absence of hypertension in the



face of marked renal failure, and (3) severe uremia caused by focal embolic glomerulonephritis

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# STUDIES ON THE HEMOLYSIS OF HUMAN ERYTHROCYTES BY HOMOLOGOUS COMPLEMENT IN THE PRESENCE OF TANNIC ACID<sup>1</sup>

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The existence of so-called artificial amboceptors, which have the property of "sensitizing" red cells for lysis by complement, has long been recognized. Landsteiner (1) showed that erythrocytes were agglutinated by small amounts of silicic acid and became hemolyzed upon the subsequent addition of complement. Reiner and Fischer (2) demonstrated that tannic acid exerted a similar action on red cells. Neufeld and Ettinger-Tulczyńska (3) confirmed this observation, and extended the list of "sensitizers" to include alum and the salts of several heavy metals. Freund (4) showed that tannic acid rendered erythrocytes more susceptible to phagocytosis. Gordon and Thompson (5, 6) reported that tannic acid and the salts of certain heavy metals enhanced the phagocytosis of staphylococci and *E. typhosus*. The mechanism by which these substances produced their effects has never been made clear. It has been assumed that they damage the cell membrane, but the nature of this damage is not understood.

Very little is known about the mechanism by which hemolysis occurs when red cells, specific antibody, and complement are combined. From a theoretical viewpoint, this reaction is of fundamental importance. Aside from its relation to problems of red cell function and destruction, the hemolytic system offers a relatively simple model with which to study the general problem of tissue injury by immune mechanisms. The deficiencies in our understanding of this problem are basic. Much has been learned about the nature and properties

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<sup>2</sup> Senior Pediatric Fellow of the National Research Council. The technical assistance of Mrs. Robert Woodyard is acknowledged.

of antibody and complement, but little is known about the actual function of these agents in the production of tissue damage

In attempting to study the rôle of complement in hemolysis, one is confined to a limited range of experimental manipulation by the complexity and variability of the reagents involved, in the system ordinarily employed, the red cells are derived from one species (sheep), complement from another (guinea pig), and the antibody from a third (rabbit) The present investigation of the action of tannic acid as an artificial "sensitizer" represents an attempt to simplify the conditions under which the lytic action of complement can be observed, by substituting a known chemical reagent for antibody, and by using homologous red cells and complement

In preliminary experiments using physiological saline solution as a diluent for all reagents, hemolysis of red cells from various species could be elicited only occasionally with tannic acid and complement, and, when lysis did occur, it was usually incomplete and required high concentrations of complement When, however, the salt concentration was decreased, a marked enhancement of the hemolytic action of tannic acid with human red cells and complement was observed, although the cells and complement of other species still yielded irregular results In view of this finding, the subsequent experiments on which this report is based were carried out for the most part with human red cells and complement using 0.7 percent sodium chloride instead of the customary physiological saline as diluent It was found that tannic acid, under the conditions to be described, caused human erythrocytes to undergo lysis with complement from the same or other human subjects It was possible to titrate human complement by this means with reproducible endpoints similar to those obtained in titrations with amboceptor-sensitized sheep cells Moreover, human red cells and complement could be employed with tannic acid as an indicator system in complement fixation tests A preliminary report of these observations has been published (7) The present paper is concerned with a detailed description of the conditions which are necessary for hemolysis with tannic acid and complement, and with certain observations which bear upon the mechanism of the phenomenon

## MATERIALS AND METHODS

*Diluent* Unless otherwise stated, the diluent for all reagents consisted of 0.7 percent sodium chloride in distilled water.

*Cells and Complement* Venous blood was obtained from normal human subjects, a portion being added to oxalate crystals and the remainder allowed to clot at room temperature. Cells from the oxalated blood were washed three times in at least 20 volumes of 0.85 percent saline and then taken up in sufficient 0.7 percent saline to make a one percent suspension by volume. The clotted blood was centrifuged at 2000 R P M for 10 minutes, and the serum so obtained kept in an ice bath until used as complement. Fresh lots of cells and complement were prepared each day.

*Tannic Acid* A single lot of crystalline tannic acid<sup>3</sup> was used in all experiments to be described. A one percent solution in 0.7 percent sodium chloride was prepared each day and various dilutions of this were tested for the optimal concentration in the hemolytic system under study.

*Sensitization of Cells* Two parts of the red cell suspension were added to one part of tannic acid and the mixture allowed to stand at room temperature for 15 minutes or longer before the addition of complement or other reagents. It was not necessary to adjust the pH of the tannic acid solution before sensitization, since the mixture of cells and tannic acid, in the concentrations usually employed, had a pH of 7.0 or slightly higher.

*Titration of Complement* Human serum was added to tubes in volumes of 0.07, 0.06, 0.05, 0.04, 0.03, and 0.02 cc and the volume was made up to 0.2 cc with 0.7 percent NaCl. 0.6 cc of sensitized cells was then added and the total volume increased to 1.2 cc by the addition of 0.4 cc of 0.7 percent sodium chloride. The tubes were then placed in a water bath at 37°C for 30 minutes after which readings of the degree of hemolysis were made. The smallest amount of complement yielding complete or four plus hemolysis was taken as the endpoint and represented one unit of complement activity. In studies of the effect of other substances on hemolysis, the additional

<sup>3</sup> C. P. Crystalline Tannic Acid, Lot No. 1, 1945 J. T. Baker Co., Phillipsburg, New Jersey

reagents were employed in volumes of 0.2 cc in place of an equivalent volume of the sodium chloride solution

# EXPERIMENTAL

## *Factors Influencing the Reaction*

*The Effect of Salt Concentration on Hemolysis* Various concentrations of sodium chloride in distilled water were employed as diluent for all reagents involved, and the endpoints of complement activity

TABLE I

*The Effect of NaCl Concentration on the Hemolysis of Human Cells by Human Complement and 0.06 Percent Tannic Acid*

NaCl IN DILUENT	VOLUME OF COMPLEMENT (CC)							CON- TROL†
	0.06‡	0.05	0.04	0.03	0.02	0.01	0.00	
<i>percent</i>								
0.60	++++*	++++	++++	++++	0	0	0	0
0.65	++++	++++	++++	++++	0	0	0	0
0.70	++++	++++	++++	++++	+++	0	0	0
0.75	++++	++++	+++	++	0	0	0	0
0.80	++++	++++	++	0	0	0	0	0
0.85	++	+	0	0	0	0	0	0
0.90	+	+	0	0	0	0	0	0

\* In this and following tables, symbols indicate +++++, complete hemolysis, +, slight hemolysis, 0, no hemolysis

† Control 0.06 cc complement with unsensitized cells

‡ Figures indicate amount of complement in final volume of 0.2 cc

determined In these experiments, tannic acid was used in a concentration of 0.06 percent, and red cells in a one percent suspension The results of a representative experiment are shown in Table I It will be seen that the optimal salt concentration for the reaction was in the vicinity of 0.7 percent Hemolysis was almost completely inhibited by sodium chloride concentrations of 0.85 or 0.9 percent, while concentrations of 0.6 percent were less effective than 0.7 percent At 0.5 percent or less, hemolysis due to hypotonicity occurred Because of these findings, the diluent employed for all reagents in the experiments to be recorded consisted of 0.7 percent sodium chloride, unless otherwise stated

*Effect of Salt Concentration on Sensitization of Red Cells* It was of interest to determine whether varying concentrations of sodium chloride exerted an effect on the capacity of tannic acid to sensitize the red cells, independent of the effect on hemolysis itself. Accordingly, red cells were suspended in different concentrations of sodium chloride and exposed to 0.06 percent tannic acid dissolved in similar salt concentrations. After the mixtures had stood together for 30 minutes, the cells were separated by centrifugation and resuspended in 0.7 percent sodium chloride. A titration of complement using these cells was then made, with the results shown in Table II. It will be seen that cells which had been exposed to tannic acid in the pres-

TABLE II

*The Effect of NaCl Concentration on the Sensitization of Human Red Cells by 0.06 Percent Tannic Acid*

NaCl IN DILUENT*	VOLUME OF COMPLEMENT (CC)						
	0.1	0.08	0.06	0.04	0.02	0.01	0.00
percent							
0.70	++++	++++	++++	++++	++++	++++	0
1.5	+	+	±	±	±	±	0

\* Red cells and tannic acid were mixed together in NaCl solution of the concentrations indicated. After 30 minutes, the cells were removed, washed, and tested with complement in 0.7 percent NaCl.

ence of 1.5 percent saline were not lysed by complement and presumably had not been sensitized, while cells which had been in an environment of 0.7 percent NaCl were hemolyzed.

These results indicated that the sensitization of red cells by tannic acid was prevented in excessive concentrations of sodium chloride. At the same time, it was demonstrated that red cells adequately sensitized with tannic acid could be separated from the tannic acid and still retain their susceptibility to lysis by complement. This property of red cells will be dealt with below, where it will be shown that an actual adsorption of tannic acid by red cells occurs.

*Effect of Glucose as Diluent for the Reagents Involved* The effect of substituting glucose solutions for NaCl as diluent for the reagents in the test was studied. Red cells which had been washed in physiological saline were resuspended in glucose solutions of 4, 5 and 6

percent and exposed to 0.06 percent tannic acid dissolved in similar concentrations of glucose. Complement diluted in similar solutions was then added. The results are shown in Table III. It will be seen that hemolysis occurred with tannic acid and complement when sodium chloride was omitted from the diluting fluid, although the activity of complement was less than in 0.7 percent saline.

The ability of tannic acid to sensitize cells in a diluent of glucose was studied. Red cells washed three times in physiological saline were suspended to one percent in 5 percent glucose. 0.06 percent tannic acid in the same diluent was added, and the mixtures allowed to stand for 30 minutes. The cells were then washed once in 5 percent glucose and resuspended in 0.7 percent sodium chloride. This

TABLE III

*Effect of Glucose on Hemolysis of Cells by Complement and 0.06 Percent Tannic Acid*

DILUENT*	VOLUME OF COMPLEMENT (CC.)					
	0.1	0.08	0.06	0.04	0.02	0.00
0.7% NaCl	++++	++++	++++	++++	0	0
4% Glucose	++++	++++	++++	0	0	0
5% Glucose	++++	++++	+++	0	0	0
6% Glucose	+++	+++	+++	0	0	0

\* All reagents prepared in diluent indicated

suspension was used in a titration of complement. Red cells so treated were as susceptible to lysis by complement as were cells sensitized in 0.7 percent NaCl showing that complete sensitization occurred in a diluent of glucose.

*The Optimal Concentration of Tannic Acid* Various concentrations of tannic acid, dissolved in 0.7 percent saline, were added to a one percent suspension of red cells, and the mixture allowed to stand for 30 minutes. Titrations of complement were then made with each of these mixtures. The results are shown in Table IV. The range of effective tannic acid was narrow, lying between 0.12 and 0.015 percent. Above or below these concentrations, hemolysis failed to occur. For the experiments to be reported, a dilution of 0.06 percent tannic acid was used unless otherwise stated.

*The Optimal Concentration of Red Cell Suspensions* Various con-



centrations of red cells in 0.7 percent saline were compared for their susceptibility to lysis in the presence of tannic acid and complement. The optimal concentration of cells was found to be one percent. Lower concentrations of cells rendered the reading of endpoints of hemolysis unreliable. The use of higher concentrations resulted in

TABLE IV

*Effect of Concentration of Tannic Acid on Hemolysis of Cells by Complement*

CONCENTRATION OF TANNIC ACID	VOLUME OF COMPLEMENT (cc)				
	0.1	0.08	0.06	0.04	0.02
<i>percent</i>					
0.1	++++	++++	++++	+	0
0.06	++++	++++	++++	++++	0
0.03	++++	++++	++++	++++	0
0.015	+++	+++	+++	++++	++
0.008	0	0	0	0	0

TABLE V

*Effect of pH on Sensitization of Red Cells by 0.06 Percent Tannic Acid*

pH*	VOLUME OF COMPLEMENT (cc)							
	0.07	0.05	0.03	0.01	0.008	0.006	0.004	0.002
6.0	++	++	+++	++	++	++	++	++
6.5	+++	+++	++++	+++	+	+	0	0
7.0	++++	++++	++++	++++	++++	+	+	0
7.5	++++	++++	++++	++++	++++	++++	0	0
8.0	++++	++++	++++	++++	++++	++++	+	0
8.5	++++	++++	++++	++++	++++	+	0	0

\* Figures refer to pH in mixtures of cells and tannic acid. Cells were separated after 30 minutes and tested with complement.

hemolysis which was usually incomplete, regardless of the amounts of tannic acid or complement.

*The Effect of pH on the Sensitization of Cells* Samples of a one percent suspension of red cells were adjusted to various pH values by the addition of 0.185 M hydrochloric acid or 0.185 M sodium hydroxide. Tannic acid solutions, adjusted to similar pH values, were added to the cells and the mixtures allowed to stand for 30 minutes. The cells were then separated by centrifugation and resuspended to the

appropriate volume in 0.7 percent saline. The susceptibility of these cell suspensions to lysis by complement was then tested with the results shown in Table V. It will be noted that the optimal pH for sensitization of red cells by tannic acid was between 7.0 and 8.0.

*The Effect of Time of Exposure on the Sensitization of Red Cells by Tannic Acid* To samples of red cell suspension were added 0.06 and 0.015 percent tannic acid respectively, and the mixtures allowed to stand for varying periods of time before being tested for hemolysis by the addition of complement. The results are shown in Table VI.

TABLE VI  
*Time Required for Sensitization of Red Cells by Tannic Acid*

CONCENTRATION OF TANNIC ACID	PERIOD OF SENSITIZATION	VOLUME OF COMPLEMENT (cc)					
		0.1	0.06	0.05	0.04	0.03	0.02
percent 0.06	1 minute		++++	++++	+	0	0
	5 minutes		++++	++++	++++	++++	+
	15 minutes		++++	++++	++++	++++	0
	60 minutes		++++	++++	++++	++++	0
	24 hours		++++	++++	++++	++++	++
0.015	1 minute	++	++	++	+	+	±
	15 minutes	++	++++	+++	++	+	+
	30 minutes	++++	++++	+++	++	+	+
	60 minutes	++++	++++	++++	++++	++++	++

With 0.06 percent tannic acid, hemolysis by the smallest effective amounts of complement was demonstrable at the end of 5 minutes, indicating that sensitization of the red cells was completed within this period. With a lesser concentration of tannic acid (0.015 percent), however, sensitization was not complete until the cells had been exposed to tannic acid for at least 30 minutes. In other experiments, similar observations were made, i.e., within the effective range of tannic acid, the rate of sensitization was slower with the lower concentrations of tannic acid. Once sensitization of red cells had occurred, the cell suspensions retained their susceptibility to lysis by complement for a period of 24 hours without change.

*The Effect of Using Complement and Cells of Different Individuals*

*and Different Species* The degree of hemolysis produced by the complement of one human subject against suspensions of sensitized cells from other individuals was tested. In cross tests between four individuals, no significant difference was observed between the hemolytic capacity of a sample of human complement for the cells of the same or other persons, or between the susceptibility to lysis of different lots of sensitized human cells.

The results of testing complement and tannic-acid treated cells obtained from other species were quite different. Human serum could not be tested satisfactorily against the red cells of rabbits or guinea pigs because of the frequent occurrence of natural lysins for these cells in human serum. Rabbit serum was completely lacking in hemolytic property for homologous cells treated with tannic acid, and produced only slight lysis of human and guinea pig cells. Guinea pig serum contained a natural lysin for human cells, and exhibited only slight hemolysis with tannic-acid treated guinea pig cells. Human, rabbit, and guinea pig serum were without significant hemolytic effect on sheep or mouse cells treated with tannic acid.

These results indicated that under the conditions employed in the test, satisfactory and reproducible hemolysis could be obtained only in the system consisting of human red cells and human complement. It is possible that the failure to demonstrate hemolysis with complement and cells from other species may be due to a greater susceptibility of the complement in these species to a primary anticomplementary action of tannic acid. Experiments which support this explanation will be described below.

*The Effect of Variation in the Order of Mixing the Reagents* In order to elicit hemolysis, it was necessary that red cells and tannic acid be allowed to stand together for a sufficient time to permit the sensitization of cells to take place. When complement was added to the cell suspension before tannic acid, hemolysis did not take place. When complement, cells, and tannic acid were mixed simultaneously, hemolysis likewise failed to occur. If small amounts of serum, or other protein solutions, were exposed to tannic acid prior to the addition of cells, sensitization of the cells was prevented. Moreover, if cells were used which had been inadequately washed, sensitization by tannic acid could not be produced. Studies on the mechanism by which serum inhibits sensitization will be described below.

*Evidence for Complement as the Agent Causing Hemolysis*

In order to be certain that the hemolysis of tannic acid-sensitized cells by serum was due to complement, and not to other lytic substances in the serum, a study of the properties of the serum factor was undertaken. The following pertinent observations were made: 1) Heating fresh serum at 56°C for 30 minutes caused complete inactivation of its lytic action. Inactivation at this temperature is a characteristic feature of complement. 2) The addition of heparin or congo red to serum prevented the lysis of sensitized cells. These substances are known to be anticomplementary. 3) Specific complement fixation by three antigen-antibody combinations was demonstrated, using human complement and red cells sensitized with tannic

TABLE VII

*Titration of Human Complement with Tannic Acid-Sensitized Human Cells and Amboceptor-Sensitized Sheep Cells*

CELLS	VOLUME OF COMPLEMENT (cc)				
	0.08	0.06	0.04	0.02	0.01
Human cells and tannic acid*	++++	++++	++++	++	0
Sheep cells and amboceptor†	++++	++++	++++	++	0

\* 0.06% tannic acid

† 4 units of rabbit amboceptor

acid as the lytic system. A detailed account of this experiment is given elsewhere (7). 4) The titer of complement in several samples of human serum was determined in simultaneous tests with a) human cells sensitized with tannic acid, and b) sheep cells sensitized with rabbit amboceptor. As is shown in Table VII, the endpoints of complement activity as determined by the two methods were indistinguishable.

The foregoing observations are regarded as sufficiently strong evidence to permit the assumption that the lysis of tannic acid-sensitized cells is due to the complement.

*The Adsorption of Tannic Acid by Red Cells*

In order to determine whether the sensitization of red cells for hemolysis by complement involved the adsorption of tannic acid by the cells, the following experiment was performed:

Two volumes of a suspension of red cells were mixed with one volume of tannic acid solution. Immediately after mixing, and at intervals of 15, 30, and 60 minutes thereafter, aliquot portions of the suspension were centrifuged at 1000 R P M for 5 minutes. The sedimented cells were taken up in NaCl solution, washed once by centrifugation, and resuspended to the original volume of the mixture. These suspensions were then tested for their susceptibility to the lytic action of complement by mixing 0.6 cc of each suspension with 0.2 cc of complement dilutions and 0.4 cc of NaCl solution. At the same time, the supernatant fluid obtained after centrifugation of each mixture

TABLE VIII

*The Susceptibility of Human Red Cells to Lysis by Complement, When the Cells were Mixed with 0.06 Percent Tannic Acid Solution and Then Removed from the Solution by Centrifugation*

PERIOD OF EXPOSURE*	VOLUME OF COMPLEMENT (cc)					
	0.1	0.08	0.06	0.04	0.02	0.01
minutes						
1	+++	+++	++	0	0	0
15	++++	++++	++++	++++	++++	+++
30	++++	++++	++++	++++	++++	++±
60	++++	++++	++++	++++	++++	++±

\* The indicated periods refer to the time of exposure of red cells to 0.06 percent tannic acid, before cells were removed by centrifugation and tested with complement.

of cells and tannic acid was tested for its capacity to sensitize untreated cells. Chemical estimations were made of the amount of tannic acid in the supernatant fluids as compared with the amount in the original solution.

As is shown in Table VIII, red cells retained their susceptibility to lysis by complement after they had been removed from the tannic acid solution and washed. The susceptibility of cells which were removed immediately after exposure to tannic acid was considerably less than that of cells which had remained in tannic acid 15 minutes or longer. This finding was in agreement with the previous observation that an optimal time of 5 to 15 minutes is required for sensitization of red cells.

The amount of tannic acid remaining in the supernatant fluid after

the removal of red cells was less than that in the original solution, and decreased the longer the cells and tannic acid were allowed to stand together. This finding is illustrated in Table IX, in which it will be seen that the capacity of the supernatant fluid to sensitize normal cells was less than that of the original tannic acid solution in each instance, with the supernatant fluid of the mixture which had been kept for 60 minutes before centrifugation showing the least ability to sensitize. Moreover, the amount of free tannic acid as determined colorimetrically<sup>4</sup> was also decreased by exposure to red cells. The supernate from the mixture kept for 30 minutes contained

TABLE IX

*The Diminution in the Sensitizing Action of 0.06 Percent Tannic Acid Solution, When Human Red Cells Were Mixed with the Solution and Then Removed by Centrifugation*

PERIOD OF EXPOSURE*	VOLUME OF COMPLEMENT (CC)				
	0.1	0.03	0.06	0.04	0.02
<i>minutes</i>					
1	++++	++++	++++	++++	0
15	+++	+++	++	++	0
30	++	++	+	+	0
60	++	+	+	0	0

\* The indicated periods refer to the time of exposure of red cells to 0.06 percent tannic acid, before cells were removed and the supernatant fluid tested with new cells and complement.

29 mg per cc as contrasted with 40 mg per cc for the original solution of tannic acid.

The foregoing observations indicated that the sensitization of red cells by tannic acid was associated with the adsorption of this substance by the cells.

#### *The Fixation of Tannic Acid by Serum*

It was found that the order in which red cells, tannic acid, and complement were combined was an important factor in the production of hemolysis. When tannic acid and red cells were mixed and allowed

<sup>4</sup> Colorimetric determinations of tannic acid were performed after the method described by Greissmayer (8).

to stand for 15 minutes or longer before addition of complement, hemolysis invariably occurred. However, when tannic acid and complement were mixed first and then added to the cells, or when all three were placed together simultaneously, hemolysis did not take place.

Two possible explanations for this observation were considered: 1) that tannic acid, before its adsorption by the cells, produced an irreversible anticomplementary effect on the serum, thus preventing the lytic action of the latter once the cells had become sensitized, or 2) that in the presence of complement, tannic acid was kept from the cells by virtue of an affinity between tannic acid and serum. Choice between these alternative explanations was based on the following experiments.

The anticomplementary effect of tannic acid was tested with human and guinea pig complement, using sheep cells sensitized with rabbit amboceptor as the hemolytic system. Physiological saline was used as diluent for all reagents. Decreasing amounts of complement in a volume of 0.2 cc. were mixed with 0.2 cc. of tannic acid in concentrations of 0.06, 0.03, 0.015, 0.008, 0.004 and 0.002 percent. The tubes were then placed in a 37°C. water bath for 30 minutes, following which sensitized sheep cells, consisting of two parts of 2 percent cells and one part of rabbit amboceptor (4 units), were added to each tube in 0.6 cc. amounts. The tubes were reincubated for 30 minutes at 37°C., and the degree of hemolysis recorded.

The results are shown in Table X. It will be seen that tannic acid in the concentrations used was only slightly anticomplementary for human complement. This anticomplementary effect did not appear sufficiently strong to account for the complete blocking of hemolysis when tannic acid, complement, and red cells were mixed simultaneously. In contrast, tannic acid was markedly anticomplementary for guinea pig complement, causing inhibition of hemolysis in concentrations as low as 0.004 percent. The latter observation may explain the failure of guinea pig serum to produce hemolysis of red cells sensitized by tannic acid, as noted above.

In order to determine whether human serum was capable of preventing the adsorption of tannic acid by red cells, the following experiment was performed.

Two parts of a suspension of red cells were mixed with one part of varying dilutions of human serum, and one part of tannic acid solution was then added. After standing for 30 minutes, portions of each mixture were tested for hemolysis by the addition of complement. The results of an illustrative experiment are shown in Table XI. It will be seen that sensitization of the cells was prevented by human serum in a dilution of 1/100 and partially inhibited by a dilution of 1/500. An inhibiting effect of similar degree was demonstrated in

TABLE X

*The Anticomplementary Effect of Tannic Acid for Human and Guinea-Pig Complement, When Tested with Amboceptor-sensitized Sheep Cells*

CONCENTRATION OF TANNIC ACID	VOLUME OF HUMAN COMPLEMENT (cc.)					
	0.06	0.05	0.04	0.03	0.02	0.01
<i>percent</i>						
0.06	++++	++	0	0	0	0
0.03	++++	++++	++++	++	0	0
0.015	++++	++++	++++	++++	+	0
0.00	++++	++++	++++	++++	+++	0
	VOLUME OF GUINEA PIG COMPLEMENT (cc.)					
	0.014	0.012	0.010	0.008	0.006	0.004
0.06	0	0	0	0	0	0
0.015	0	0	0	0	0	0
0.008	+++	+	0	0	0	0
0.004	++++	++++	+++	+	0	0
0.002	++++	++++	++++	++	+	0
0.00	++++	++++	++++	+++	++	0

serum which had been heated at 72°C for 30 minutes, and also in a 0.1 percent solution of crystalline egg albumin.

The red cells contained in mixtures of tannic acid and serum were removed by centrifugation, washed, resuspended in NaCl solution, and tested with complement. Hemolysis of these cells did not occur. The concentration of tannic acid in the supernatant fluid after removal of the cells, determined colorimetrically, remained the same as before exposure of the cells.

The foregoing observations demonstrated that the adsorption of



tannic acid by red cells was blocked by small amounts of serum. It is probable that this effect, rather than an anticomplementary action by tannic acid, explains the failure of hemolysis to occur when cells, complement, and tannic acid were mixed simultaneously. The mechanism which underlies this blocking effect has not been determined, but it seems probable that tannic acid may form a union with a component of serum and thus be withheld from adsorption by the cells.

TABLE XI

*The Inhibitory Effect of Human Serum on the Sensitization of Red Cells by 0.06 Percent Tannic Acid, When Serum and Cells Were Combined before the Addition of Tannic Acid*

SERUM DILUTION*	VOLUME OF COMPLEMENT (cc)	
	0.1	0.04
1:10	0	0
1:100	0	0
1:200	++	0
1:500	+++	++
1:1000	++++	++++
No serum	++++	++++

\* Figures refer to dilution of serum mixed with red cells before the addition of tannic acid.

#### *Attempts to Demonstrate the Adsorption of Complement by Sensitized Cells*

It has been shown that tannic acid may possess a dual affinity for the two participants in the hemolytic reaction, the red cell and the serum. It is adsorbed by the red cell and withheld from adsorption in the presence of serum. The latter effect is not specific for complement, since it is also a property of heated serum and egg albumin. It seemed possible that the action of tannic acid might be the result of its dual attraction for complement or a component of complement, and red cell. Therefore, the following experiments were undertaken in order to determine whether red cells which had been sensitized with tannic acid were capable of adsorbing complement.

20 cc of a one percent suspension of red cells were mixed with 10 cc of 0.06 percent tannic acid and allowed to stand for 30 minutes. The cells were then removed by centrifugation and taken up in one

cc of chilled human serum. The mixture was kept in an ice bath for 18 hours, centrifuged in the cold, and the supernate compared for complement activity with an untreated sample of the same serum and with a sample mixed with unsensitized cells.

No decrease in the activity of complement after exposure to sensitized cells could be demonstrated, indicating that adsorption of complement by the sensitized cells did not take place to a degree detectable by this method.

The effect of exposing sensitized cells to complement in the cold on their susceptibility to hemolysis was studied.

A suspension of sensitized cells was prepared as in the preceding experiment and chilled in an ice bath. Aliquots of 0.6 cc were mixed with 0.2 cc of chilled complement diluted 1:2, and the mixture kept in an ice bath for one hour. The cells were then separated by centrifugation in the cold, and resuspended in 1.2 cc of 0.7 percent NaCl. As long as these suspensions were kept cold, lysis did not occur. When they were warmed to 37°C, however, complete hemolysis took place within 10 minutes. Sensitized cells which were treated in a similar fashion with heat-inactivated complement did not hemolyze.

This observation indicated that the sensitized cells had either adsorbed a sufficient amount of complement to cause hemolysis when they were warmed, or that there remained enough complement in the fluid surrounding the sedimented cells to cause hemolysis. In order to settle this point, the effect of washing sensitized cells following exposure to complement was studied.

Sensitized cells were treated with complement in the cold as in the preceding experiment, centrifuged, and washed two times in cold NaCl solution. They were suspended in NaCl solution and placed in a 37°C water-bath for 30 minutes. Hemolysis did not occur. These cell suspensions were then tested for their susceptibility to complement and compared with sensitized cells which had been treated in the cold either with NaCl solution alone or with serum heated at 56°C for 30 minutes. Falling dilutions of complement were added, in 0.2 cc amounts, to 1.0 cc of cell suspensions, and the tubes placed in the water-bath for 30 minutes.

The results of this experiment are shown in Table XII, in which the

endpoint of complement activity is indicated in terms of the final dilution of complement in each tube. It will be seen that the sensitized cells which had previously been treated with cold complement were much more susceptible to the action of complement than cells treated with NaCl solution or heated serum. For washed sensitized cells alone, the end point of complement activity was in a final dilution of 1:240. After being exposed to cold complement and then washed, these cells were hemolyzed by dilutions of complement as high as 1:1920. No hemolysis was produced by serum which had been heated at 56°C for 30 minutes.

TABLE XII

*The Increased Susceptibility of Human Red Cells to Lysis by Complement, When the Cells Were Sensitized with 0.06 Percent Tannic Acid, Treated with Complement in the Cold, Washed, and Then Tested with Complement at 37°C*

CELLS*	VOLUME OF COMPLEMENT (CC)							
	0.02	0.01	0.005	0.002	0.001	0.0005	0.0001	0
Treated with cold complement	++++	++++	++++	++++	++++	++++	0	0
Untreated	++++	++++	++++	0	0	0	0	0

\* See text for method of treatment

In an attempt to find an explanation for the heightened susceptibility of these cells, the effects of the two components of complement, i.e., "mid-piece" and "end-piece," were studied. Fresh human serum was fractionated by the CO<sub>2</sub> method described by Ecker and Pillemer (9), and each component was made up in 0.7 percent NaCl. Sensitized cells which had previously been exposed to cold complement and washed, as in the preceding experiment, were tested with varying dilutions of mid-piece and end-piece. It was found that complete hemolysis was produced to an equal degree by each component alone, in final dilutions which were the approximate equivalent of a 1:120 dilution of whole serum. Neither mid-piece or end-piece, acting alone, were capable of causing hemolysis in sensitized cells which had not been treated with cold complement, although a combination of these components produced complete hemolysis.

## DISCUSSION

It has been shown that tannic acid has an effect on human erythrocytes which is, in its end-result, analogous to the action of a specific antibody. Red cells which are exposed to tannic acid become susceptible to lysis by complement from the same or other human individuals. By this method it is possible to perform titrations of human complement with endpoints similar to those obtained when complement is determined in the usual manner with amboceptor-sensitized sheep cells. Moreover, as shown in another communication (7), this hemolytic system can be employed as the indicator in certain types of complement fixation tests.

Certain conditions are necessary before the hemolytic reaction with tannic acid can be elicited with reproducible results. The concentration of sodium chloride is of the first importance. The reaction may be partially or even completely inhibited if physiological saline solution is used as the diluting fluid for the reagents, and it is necessary to use less concentrated salt solutions. In the present study the optimal sodium chloride concentration was found to be 0.7 percent. Higher concentrations inhibit the hemolytic reaction in two ways: tannic acid is prevented from preparing the red cells for lysis, and complement is prevented from lysing cells which have already been prepared with tannic acid. It is of interest that hemolysis occurs when isotonic glucose is substituted for sodium chloride in the diluting fluid, although complement is less effective in glucose than in 0.7 percent sodium chloride.

The order of mixing the reagents employed in the reaction is also of importance. It has been shown that the red cells and tannic acid must be placed together first, and a period of at least 5 minutes allowed for sensitization of the cells before the addition of complement. If complement is present at the time when cells and tannic acid are combined, hemolysis does not occur. Relatively small amounts of fresh or heated serum, or albumin solution, prevent the sensitization of red cells by tannic acid. The probable explanation for this effect is that the tannic acid combines with the soluble protein and is thus withheld from adsorption by the cell.

The optimal concentration of tannic acid was found to lie between 0.12 and 0.015 percent. Within this narrow range, the time required

for the sensitization of red cells varies inversely with the concentration of tannic acid. The optimal concentration of red cells is a one percent suspension of washed cells. The optimal pH for sensitization of red cells is between 7.0 and 8.0.

That the factor in human serum which caused hemolysis is actually complement is indicated by the following findings: the factor is completely inactivated by heating at 56°C for 30 minutes, and by two known anticomplementary agents, heparin and congo red. It is fixed in the presence of antigen-antibody combinations, as has been shown in another paper (7). Finally, when human serum is tested for complement activity simultaneously with tannic-acid-sensitized human cells and amboceptor-sensitized sheep cells, the endpoints are identical.

The mechanism of action of tannic acid is not known. The effects of this substance on tissues in general have been attributed to its capacity to precipitate soluble proteins, to its dehydrating property, or to its ability to unite chemically with proteins. Olitsky and Cox (10) have shown that the intranasal instillation of tannic acid in mice causes an increased resistance to intranasally introduced equine encephalitis virus, which was attributed to a direct effect of tannic acid on the nasal mucosa. Recently, Green (11) has demonstrated that tannic acid has the property of inhibiting influenza virus *in vitro* and *in vivo*, presumably due to an action of this substance on the virus itself.

The results of the present study indicate that tannic acid may combine with some component of the red cell. When this union has been established, the cells may be washed repeatedly without loss of their susceptibility to complement. Moreover, when the red cells are added to tannic acid solution and then removed by centrifugation, the solution loses some of its capacity to sensitize new cells and the concentration of tannic acid in the solution, as determined colorimetrically, becomes decreased. These observations suggest that tannic acid is adsorbed to the red cells.

Once tannic acid has united with the red cell, it may act as a source of attraction for complement or for some component of complement, thus making it possible for the latter to be brought to the cell surface. An actual adsorption of complement by sensitized cells has not been

demonstrated, but suggestive evidence for this was offered by experiments in which it was shown that when sensitized cells were mixed with complement in the cold and then removed from complement washed, and resuspended in sodium chloride solution, they were susceptible to a much smaller amount of complement than is ordinarily required for the hemolysis of sensitized cells. Moreover, these cells were lysed when either mid-piece or end-piece were added alone, although neither of these components was capable by itself of lysing sensitized cells which had not been treated with complement. Heat-inactivated serum had no effect on these treated cells. These results suggest that during the time of exposure of sensitized cells to complement in the cold, some portion of complement may have been taken on the cell, so that subsequently the addition of a very small amount of complement was sufficient to cause hemolysis. No explanation can be given for the capacity of both mid-piece and end-piece to bring about hemolysis in this case, although it is possible that each of the fractionated components may have contained residual traces of the ether.

An alternative explanation for the heightened susceptibility of sensitized cells following treatment with complement in the cold is that the cells were actually injured in some fashion during the period of exposure to complement, although this injury was not sufficient to cause hemolysis. Subsequent exposure of the cells to complement at 37° C might then have resulted in hemolysis with smaller amounts of complement than are usually necessary.

It is of interest that the hemolytic reaction with tannic acid and complement could be elicited reproducibly only with the homologous system involving human cells and serum. Attempts to produce hemolysis with cells and complement from rabbit, guinea pig, sheep and mouse sources were unsuccessful. In the case of the guinea pig, it has been shown that the failure may have been due to a primary anticomplementary effect of tannic acid on guinea pig complement. Attempts to demonstrate the reaction between cells from one species and complement from another yielded essentially negative results. Hemolysis sometimes occurred, but was usually incomplete and required high concentrations of complement. It is not known why human cells and complement should provide the most favorable system for the study of this phenomenon.

The results of this study do not provide information which bears directly on the problem of the mode of action of complement, but the method may be of use in an approach to this problem. The double affinity of tannic acid for the red cell and for serum protein suggests the possibility that this substance may act by bringing complement (or a component of complement) into close relationship with the red cell surface. It has been suggested by previous workers (12) that the hemolytic action of complement may be analogous to that of a surface-active agent. Further studies on this possibility are in progress.

#### SUMMARY

1 Treatment of human red cells with tannic acid rendered them susceptible to hemolysis by fresh human serum from the same or other individuals. Demonstration of this reaction depended upon the presence of optimal concentrations of sodium chloride (0.7 percent), tannic acid (0.12 to 0.015 percent), and red cells (one percent). The optimal pH range lay between 7.0 and 8.0.

2 This reaction could not be demonstrated with cells and serum from rabbit, guinea pig, mouse or sheep sources.

3 It was necessary to allow the cells and tannic acid to stand together for at least five minutes before the cells became completely sensitized. Longer periods were required when lower concentrations of tannic acid were employed.

4 When titrations of human complement were performed simultaneously with tannic acid-sensitized human red cells and amboceptor-sensitized sheep cells, the endpoints of complement activity were identical.

5 The hemolytic factor in human serum was inactivated by heating at 56°C, and by the addition of heparin or congo red.

6 Sensitization of red cells by tannic acid failed to occur if serum was present at the time when cells and tannic acid were mixed.

7 Red cells which were sensitized by tannic acid could be washed without losing their susceptibility to hemolysis by complement. Exposure of a tannic acid solution to red cells resulted in a diminution in the capacity of this solution to sensitize new red cells, as well as in a decrease in the concentration of tannic acid estimated colorimetrically.

8 When tannic acid-sensitized cells were exposed to complement in

the cold, and then washed, they became susceptible to hemolysis by much smaller quantities of complement. Such cells were also hemolyzed by complement mid-piece or end-piece alone. They did not lyse spontaneously, nor in the presence of heat-inactivated complement.

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# A SIMPLE VOLUMETER FOR MEASURING THE OXYGEN CONSUMPTION OF SMALL ANIMALS<sup>1</sup>

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WITH THE TECHNICAL ASSISTANCE OF M B GLASS AND E LEAKINS

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Although a variety of methods exist for measurement of oxygen consumption of small animals (1, 2), the apparatus to be described was designed to meet the requirements of simplicity of construction, availability of materials, accuracy and speed of operation. The principle, which reflects the 100-year-old method of Regnault and Reiset (6), depends upon measurement of the rate of change in volume in a closed-circuit metabolism chamber at constant temperature and pressure. The innovation of the present method is that volume change is determined by means of a coiled volumeter burette which is maintained at constant temperature in the bath that houses the metabolism chamber.

## APPARATUS

A conventional *Warburg-Barcroft constant-temperature bath* with stirrer is employed with a thermoregulator sensitive to 0.05°C and set to 28.0°C.

In one end of the bath is seated the *metabolism chamber*, a rectangular brass box, open at the top, 28 cm long  $\times$  20 cm wide  $\times$  13 cm deep (Figure 1). The box is supported on legs to permit circulation of bath water over all six faces, and the underside is weighted with about 9 kg of lead plate to prevent flotation. A short brass tube, 1 cm internal diameter, pierces one long side of the box. The lid of the box is a Lucite plate, 30  $\times$  30  $\times$  1 cm, with a 5 mm-deep milled slot on the under surface to permit an air-tight petrolatum seal when fitted in place. Since the Lucite sheet is larger than the base of the box, a projecting

<sup>1</sup> Work done under a contract between the Office of Naval Research, U S Navy Department and the Johns Hopkins University

shelf is available for support of the volumeter to be described presently. The Lucite is secured to the box by five steel springs distributed on three sides. In the Lucite are bored three holes, 12 mm in diameter tapered to fit No. 1 rubber stoppers and placed as indicated in the figure. These holes permit passage into the chamber of a stirrer, a thermometer and an outlet tube with a glass stopcock.

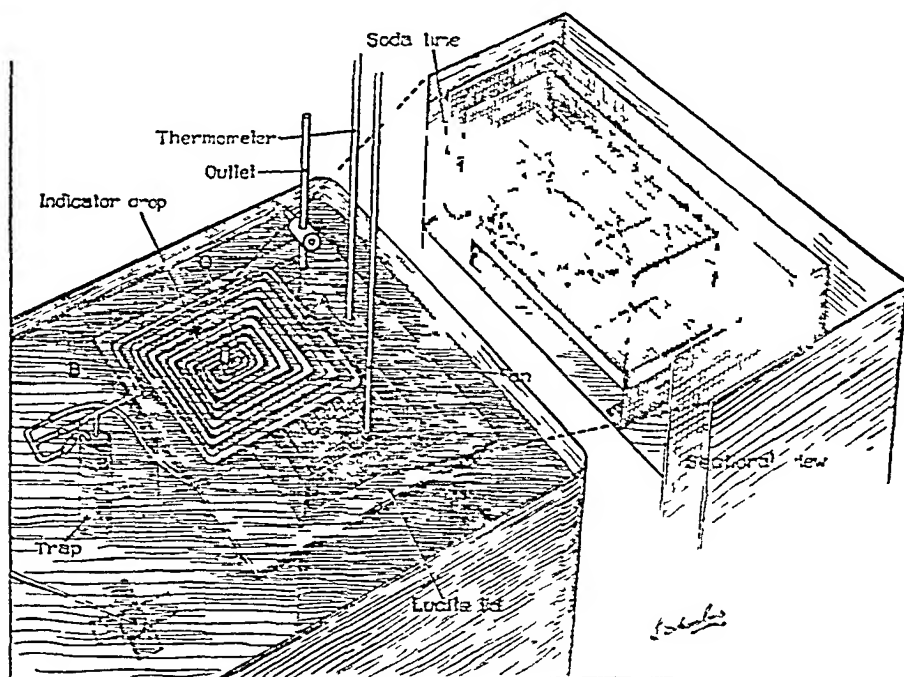


FIG 1

The chamber contains soda lime, a humidifier and the animal cage. Soda lime is contained in a U-shaped trough of No. 8 mesh metal screening which lines three sides of the box and embraces the animal cage. On the floor of the box, beneath the animal cage is placed a watch glass containing several grams of anhydrous calcium chloride granules moistened with a little water. This agent maintains chamber humidity at about 30 per cent (5). The animal cage is of No. 8 mesh wire screen with a false floor of No. 2 mesh screen providing maximum animal space of 7 x 18 x 5 cm. Effective length of the cage can be shortened by a sliding end wall, secured by a wooden pin. Below the

false floor is a removable brass tray, lined with filter paper, moistened with approximately 0.01 N HCl to neutralise urinary ammonia

Placed flat on the Lucite shelf is the *coil volumeter* made of 4 mm internal diameter Pyrex glass tubing, silicone-lined (Drifilm, #9987, General Electric Co) to prevent dispersion of the indicator drop. The tubing is approximately 3 m long, contains 7 coils, and in its outer dimensions approximates a square 20 cm wide. The central end of the volumeter curves to a perpendicular with respect to the plane of the coil and serves as an air inlet. The volumeter tube terminates as a T-tube, one arm is connected by rubber tubing to the brass tube in the side of the metabolism chamber, the other arm leads to a trap for the volumeter indicating fluid, to be described. The volumeter is kept in place by four sets of pins screwed into the Lucite top. The volumeter is marked at three points and calibrated. At 28°C, bath temperature, the volume between the initial point, O, and the intermediate point, A, is of the order of 30 ml, between O and the terminal point, B, 35 ml. These volumes were selected to provide metabolism periods of some five to 10 minutes for normal rats weighing about 200 g.

Circulation within the chamber is maintained by a fan (1 x 5 cm) driven at 1200 r.p.m. by an electric stirring motor, mounted independently above the chamber. The shaft of the stirring fan, made of stainless steel, passes into the chamber through a 5 cm long, smoothly machined brass bushing seated in a rubber stopper. This seal does not leak under pressures exceeding 10 mm Hg. Under these conditions the chamber temperature approximates 29.0°C.

The chamber is illuminated by a fluorescent lamp suspended approximately 18 inches above the bath to prevent local heating.

#### TECHNIC AND RESULTS

The animal is placed in the cage, the cage size adjusted to confine it in a comfortable space which just permits turning. Soda lime, moistened calcium chloride and the animal cage are placed within the chamber. The Lucite lid, greased appropriately, is set in place and fixed with springs. The volumeter is attached. The thermometer, the outlet tube and the stirrer are assembled.

With the outlet tube open, the chamber is gassed with oxygen through the open end of the volumeter. It is desired only to obtain

an atmosphere sufficiently rich in oxygen to permit observation of the animal for several hours without the supervision of anoxia. After appropriate gassing, the outlet tube is closed and the bath is filled with water to a level about 2 cm above the chamber lid. The graduated portion of the volumeter is, therefore, submerged, only the air inlet is above water.

At least 30 minutes are allowed for thermal equilibration and to permit the animal to fall asleep. Then a 'drop' of water, approximately 0.15 ml, colored with methylene blue, is introduced into the inlet of the volumeter to seal the circuit. Carbon dioxide evolved by the animal is absorbed by soda lime. Pressure within the chamber decreases, therefore, as oxygen is consumed. *Since the barrier between atmospheric and chamber pressures is freely movable, continuous equilibration to barometric pressure occurs, the 'drop' flows in the volumeter toward the chamber.* The time occupied by the 'drop' in passing between the graduations of the volumeter (i.e., the rate of decrease in chamber volume) is timed with a stopwatch. From this measurement is calculated the rate of oxygen consumption of the animal.

Since the animal is under observation through the Lucite top, it is possible to exclude observations obtained during and after periods of activity. Furthermore, by reading the chamber thermometer at the beginning and end of each run, it is possible to use only those periods during which temperature is constant.

Under these operating conditions the rat usually falls asleep within 20 minutes. When equilibration is complete, as signalled by constancy of the chamber temperature, readings are begun. The elapsed times for a 'drop' indicator to move from O to A and to B are measured by a stopwatch. This constitutes an internal check on the measurements. In 99 such measurements the mean difference between A and B readings was 0.02 ml O<sub>2</sub>/min/100 g rat (maximum difference 0.08, standard deviation 0.0185). At least three such complete measurements are made during periods of sleep, and the mean of these values is taken as the "basal" oxygen consumption for the experiment. Under usual circumstances a complete determination requires from 60 to 90 minutes.

As tests of reliability of the apparatus, 33 such complete determinations of oxygen consumption were performed on a litter of eight male rats of mixed albino-Wheeler strain, approximately 90 days old and

false floor is a removable brass tray, lined with filter paper, moistened with approximately 0.01 N HCl to neutralise urinary ammonia

Placed flat on the Lucite shelf is the *coil volumeter* made of 4 mm internal diameter Pyrex glass tubing, silicone-lined (Drifilm, #9987, General Electric Co) to prevent dispersion of the indicator drop. The tubing is approximately 3 m long, contains 7 coils, and in its outer dimensions approximates a square 20 cm wide. The central end of the volumeter curves to a perpendicular with respect to the plane of the coil and serves as an air inlet. The volumeter tube terminates as a T-tube, one arm is connected by rubber tubing to the brass tube in the side of the metabolism chamber, the other arm leads to a trap for the volumeter indicating fluid, to be described. The volumeter is kept in place by four sets of pins screwed into the Lucite top. The volumeter is marked at three points and calibrated. At 28°C, bath temperature, the volume between the initial point, O, and the intermediate point, A, is of the order of 30 ml, between O and the terminal point, B, 35 ml. These volumes were selected to provide metabolism periods of some five to 10 minutes for normal rats weighing about 200 g.

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As tests of reliability of the apparatus, 33 such complete determinations of oxygen consumption were performed on a litter of eight male rats of mixed albino-Whelan strain, approximately 90 days old and

200 to 250 g in weight All determinations were performed after a fast of 17 to 22 hours

Triplicate determinations (i e , three separate runs through the coil during a single residence in the metabolism chamber) agreed with a mean deviation of  $\pm 3.16$  per cent (range 0 to 10.2 per cent, S D  $\pm 2.6$  per cent) The average of triplicate determinations was recorded as the "basal" oxygen consumption This value was calculated on at least three occasions for each animal, and the arithmetic average of these measurements was recorded as the mean "basal" oxygen con-

TABLE 1

*O<sub>2</sub> Consumption of 8 Sibling Male Rats*

All values expressed as ml O<sub>2</sub>/min /100 g body weight

	RAT								GROUP
	1	2	3	4	5	6	7	8	
No of Determinations	4	3	4	6	5	3	3	5	33
Maximum	1 87	1 84	1 95	1 89	2 03	1 99	1 85	2 06	2 06
Minimum	1 76	1 77	1 67	1 67	1 75	1 85	1 76	1 67	1 67
Mean	1 82	1 80	1 83	1 78	1 87	1 90	1 80	1 83	1 83
S D									$\pm 0.036$

sumption In the series of eight litter-mates, the mean basal oxygen consumption was 1.83 ml O<sub>2</sub> STPD/min /100 g rat (Table 1)

## CALCULATION

It is assumed that gas within the volumeter is saturated with water vapor, due to the advancing 'drop', and that its temperature is that of the water bath On the other hand, gas within the chamber is unsaturated and may be warmer, due to imperfect dissipation of animal heat to the bath However, given the chamber temperature and vapor pressure, it is possible to convert the measured volume in the volumeter to the actual volume of oxygen consumed by the animal in the chamber Finally, this volume may be expressed in terms of standard temperature and pressure, dry

Chamber aqueous vapor pressure is assumed from the following considerations Vapor pressure above saturated CaCl<sub>2</sub> · 6H<sub>2</sub>O at

24–25°C is given as 7.15 mm (5). It is assumed that it is 8 mm at 28° to 30°. Since the chamber contains excess soda lime with very low vapor pressure, chamber vapor pressure is somewhere between 0 and 8 mm. Because moistened filter paper does not dry appreciably within the chamber it is suspected that chamber vapor pressure is nearer 8 than 0 mm and is assumed, therefore, to be 6 mm. Sample calculations indicate that an error of 4 mm in estimating the aqueous vapor pressure will introduce an error of less than 0.5 per cent in the final calculation.

The volume of oxygen consumed,  $V_1$  (STPD), is calculated from the calibrated volumeter,  $V$ , by applying the equation (3)

$$V_1 = V \left( \frac{1}{1 + 0.00367t} \right) \left( \frac{P - e}{760} \right)$$

where  $t$  = chamber temperature (°C),  $P$  = corrected barometric pressure (mm Hg), and  $e$  = aqueous vapor pressure = 6 mm Hg.

The rate of oxygen consumption is calculated by dividing  $V_1$  (ml) by the time (min) required for its consumption. Finally, the rate of oxygen consumption is expressed with reference to body weight (ml O<sub>2</sub>/min/100 g rat).

#### SOURCES OF ERROR

The validity of the observations depends upon maintenance of constant pressure and temperature.

Changes in barometric pressure during the traverse of the indicator 'drop' through the volumeter (5–10 min) are insignificant under usual laboratory conditions, for a 5 mm variation in barometric pressure alters the apparent volume by less than one per cent. It was assumed that the system was frictionless, and that the flow of the indicator 'drop' did not hinder instantaneous equilibration of atmospheric and chamber pressures, for pressure differentials of less than 2 mm of water produced immediate movement of the indicator 'drop'. The reliability of these assumptions was supported by close agreement between rates of oxygen consumption calculated from the speed of the same 'drop' traveling along the volumeter from O to A and from O to B.

With respect to temperature, it can be calculated that, in the apparatus described, a change in chamber temperature of 0.05°C would



produce an error of about 5 per cent, due to thermal alteration of chamber gas pressure. By using a precision chamber thermometer which was sensitive to changes of less than  $0.05^{\circ}\text{C}$ , it was possible to eliminate the occasional period in which fluctuations in chamber temperature exceeded that value.

Rate of absorption of carbon dioxide by soda lime is a function of the circulation rate of gas within the chamber. Morrison has developed an expression which describes this relationship (4) and has pointed out that a circulation rate of 1.0 per minute requires a metabolism period of no more than five minutes in order that delay in the absorption of carbon dioxide by the soda lime not introduce a lag error. By the introduction of smoke and of carbon dioxide into the chamber, and observation of movement and rate of uptake, respectively, it was estimated that the circulation rate in the chamber described here exceeds 10. Despite this rapid movement of air the rats appear comfortable and sleep.

#### SUMMARY

1) A closed-circuit, constant temperature, constant pressure apparatus is described for measurement of oxygen consumption of small animals. It offers simplicity of construction from readily available materials and speed of operation.

2) Triplicate determinations of basal oxygen consumption in the rat agreed within  $\pm 3.2$  per cent.

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# THE CLINICAL RESULTS OF COMBINED PENICILLIN AND ICE THERAPY\*

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Smith (1) showed that infection of an extremity could be treated successfully by chilling the affected part for a prolonged period

Thompson and Trumpter (2), while searching for a method to prolong the blood level of a single injection of penicillin, found that ice applied to the area injected caused slow absorption of the drug into the blood stream

The efficacy of penicillin in the treatment of certain infections is so well established that any mention of its history is unnecessary

It seemed reasonable to presume that, if an infected area was chilled and then locally injected with penicillin, a high concentration of penicillin could be localized at the site of infection for a long period of time. It also seemed possible that an infected extremity could be treated more efficaciously by a combination of cold and penicillin than by penicillin or ice alone. With this in mind, the following group of experiments were initiated

## EXPERIMENT I

Includes 15 cases of secondarily infected varicose ulcers of the lower extremity. These 15 cases were divided into three groups, designated Ia, Ib, and Ic, respectively. Each group contained one severe and four moderately severe ulcers. In each case, the ulcer had persisted for at least three months, and had been treated for at least one month with such general measures as bed rest and elevation of the affected extremity, supplemented by local treatment at the site of the ulcer with hydrogen peroxide and 5% sulfadiazine ointment. In all cases cultures showed the presence of *beta hemolytic Streptococcus* and *hemolytic Staphylococcus aureus*, in addition to other organisms.

\* Appreciation is expressed to M M Wintrobe, M D, for his suggestions in regard to the manner of presentation of this manuscript

*Procedure and Results*

Group I *a* The 5 patients in this group were treated by wrapping the extremity in two face towels and placing five ice bags on the leg for a period of five days

In the severe case of this group, pain immediately disappeared and after 3 5 days of treatment, evidence of secondary infection slowly subsided The ice bags were removed at the end of five days However, on the tenth day a recrudescence of symptoms occurred and penicillin therapy was necessary to complete cure

SEVERE	MODERATE					MILD					AV HOSP. (DAYS)
DAYS	2	4	6	8	10	12	14	16	18	20	
A- Ice Alone											38
1 SEVERE CASE	■					■					
4 MOD SEVERE											
B Penicillin Only											25
1 SEVERE CASE	■	■									
4 MOD SEVERE	■	■	■								
C Penicillin, Ice											20
1 SEVERE CASE	■	■									
4 MOD SEVERE	■	■	■								

EXPERIMENT I 15 CASES OF VARICOSE ULCERS WITH SECONDARY INFECTION

The 4 moderately severe cases were treated in the same manner as the above In these cases, however, the infection became minimal, on the average, on the ninth day and a recrudescence of symptoms appeared on the twelfth Penicillin therapy was then instituted as in the above

Patients of Group I *a* were discharged when epithelialization of the ulcer was complete and patients were without symptoms *Average hospitalization after ice therapy was initiated was thirty-eight days* NOTE, however, that cure was not complete without additional penicillin therapy

Group I *b* The 5 patients in this group received penicillin intragluteally—50,000 units, every three hours for a total of 12 to 16 million units

In the severe case of this group, all manifestations of secondary infection had disappeared by the end of the fourth day <sup>1</sup>

In the 4 moderately severe cases all manifestations of secondary infection had vanished within 3 5 to 6 days—5 days on the average

There was no recrudescence of secondary infection in patients of Group I *b*. Again the patients were discharged after the ulcer was epithelialized and the patients without symptoms. *Average hospitalization after therapy was initiated was twenty-five days*

Group I *c*. In the 5 cases of this group, ice was placed about the site of the ulcer for one hour. The area was then prepared with tincture of merthiolate, and 500,000 units of penicillin, dissolved in 10 cc of 1% novocain, was infiltrated about the site of the ulcer, and five ice bags were kept about the ulcer for a period of 5 days

In the severe case in this group, manifestation of secondary infection had disappeared by the end of the second day

Of the 4 cases of moderate secondary infection, all manifestations of secondary infection in 2 cases, had disappeared before the end of the second day and in 2 at the end of the first day

There was no recrudescence of infection in this group and *average hospitalization after therapy was initiated was 20 days*

## EXPERIMENT II

Forty-five cases of secondarily infected epidermophytosis were divided into three groups, designated II *a*, II *b*, and II *c*, respectively. Each group contained 3 patients with severe secondary infection. Infecting organisms, streptococci and staphylococci. No cases in which the infecting organism was a gram negative bacillus were considered

### *Procedure and Results*

Group II *a*. Fifteen cases were treated by wrapping the infected extremity in two face towels and placing ice bags on the leg for twelve hours

In the 3 severe cases of this group all manifestations of infection had disappeared by the end of the sixth day

In the 7 moderately severe cases, all manifestations of infection had likewise disappeared by the end of the sixth day

<sup>1</sup> Such as fever, redness, swelling, and edema

In the 5 mild cases, the infection was cleared up at the end of the second day *Average hospitalization, 12 days*

Group II *b* The fifteen patients of this group received 30,000 units of penicillin intragluteally, q 3 h for a total of 240,000 units

In the 3 severe cases of infection in this group, 1 patient became asymptomatic at the end of the fourth day of treatment, while the remaining two became asymptomatic at the end of 24 hours

In the 7 patients with moderate infection, 4 became asymptomatic at the end of the third day, while the remaining 3 required only 24 hours to be entirely free of symptoms

SEVERE	MODERATE				MILD					AV HOSP (DAYS)
DAYS	1	2	3	4	5	6	7	8	9	10
<b>A Ice Alone</b>										
3 Severe										
7 Moderate										
5 Mild										
<b>B Penicillin Only</b>										
3 Severe										
7 Moderate										
5 Mild										
<b>C Penicillin &amp; Ice</b>										
3 Severe										
7 Moderate										
5 Mild										

EXPERIMENT II 45 CASES OF INFECTED EPIDERMOPHYTOSIS

Of the five mild cases, all were asymptomatic at the end of 24 hours. However, in this group, 4 of the 15 cases developed a recrudescence of symptoms as soon as the penicillin was stopped and it was necessary to complete cure *Average hospitalization, 8 days*

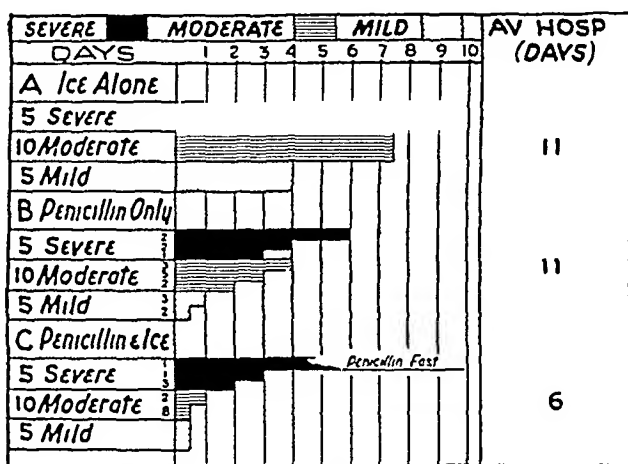
Group II *c* The 15 patients of this group received local penicillin and ice treatment as described under I *c*

All 15 cases of this group became asymptomatic within 12 hours and there was no additional treatment necessary in order to control secondary infection *Average hospitalization, 5 days*

### EXPERIMENT III

Sixty cases of wound infection of the extremities were divided into three groups containing twenty patients in each group, designated

III *a*, III *b*, and III *c*, respectively Each group contained 5 patients with marked toxic symptoms, such as hyperpyrexia, marked redness and tenderness about the wound, and regional lymph node tenderness, ten patients showing a moderate febrile response and a moderate amount of tenderness and regional lymph node tenderness, and five patients showing only mild symptoms The five cases in each group compared fairly well in regards to length, depth, and irregularity of cut surface, with the wounds of patients in each of the other groups The



EXPERIMENT III 60 CASES OF WOUND INFECTION OF EXTREMITIES

infecting organisms were usually streptococci None of the infections were produced by gram negative bacilli

### Procedure and Results

Group III *a* The twenty patients of this group received ice treatment over a period of five days

In the five severe cases, manifestations of infection disappeared within a period of nine to ten days

In the 10 moderately severe cases, manifestations of infection disappeared within seven to eight days

In the five mild cases, all signs of infection cleared up within three to five days *average hospitalization, 11 days*

Group III *b* The twenty patients in this group received intraglutely, 50 000 units of penicillin every three hours until symptoms of

infection had remained absent for twenty-four hours Total dosage, 500,000 to 2 5 million units

In the five severe cases of this group, infection disappeared at the end of the sixth day in two cases, at the end of the fourth day in two cases, at the end of the third day, in one case

In the ten cases of moderately severe infections, 3 cases were asymptomatic by the end of the fourth day, five by the end of the third day, and two by the end of the second day

In the five mild cases, three were asymptomatic by the end of the first day, and two, twelve hours after initiation of treatment *Average hospitalization, 11 days*

Group III c In the twenty cases in this group, ice was placed about the wound for one hour Then the area was prepared with tincture of merthiolate and 500,000 units of penicillin, dissolved in 10 cc of 1% novocain, were infiltrated about the wound and five ice bags were kept about the wound for a period of twelve hours to two days

One case of severe infection was apparently penicillin fast Among the four remaining cases of severe infection, one became asymptomatic by the end of the third day and three by the end of the second day

Of the ten cases with moderate infection, two became asymptomatic in twenty-four hours and eight became asymptomatic in twelve hours In the five mild cases, all were asymptomatic twelve hours after initiation of treatment *Average hospitalization, excluding one which did not respond to treatment, was six days*

#### DISCUSSION

In the series of cases presented, ice-penicillin therapy proved decidedly superior therapeutically to either interrupted intragluteal penicillin or ice The advantages are enumerated as follows

- 1 Hospitalization decidedly less
- 2 More rapid disappearance of the signs and symptoms of infection
- 3 Lower total dosage of penicillin to effect a cure
- 4 Disappearance of pain on chilling (Pain also vanishes with ice alone)

The reason for such excellent results is a matter of interesting conjecture The mechanism of the action of penicillin is unknown, but it has been suggested by Chain and Duthie (3) that penicillin is part of

an enzyme or activates an enzymatic system. Hobby (4) reported that the greatest destruction of organisms occurs during the phase of rapid multiplication. Chain and Duthie (3) lend additional creditability to Hobby's findings when they reported that during the resting of the culture cycle of the staphylococcus, large concentration of penicillin had no effect on the rate of oxygen uptake. During the earlier lag phase and the logarithmic phase of growth, penicillin exerted a strong inhibitory effect on oxygen uptake. It therefore seems likely that penicillin interferes with a metabolic function involved in the early stages of bacterial development. In view of the above, the clinical results of the ice-penicillin therapy seem paradoxical. Lee, *et al* (5) found that if cultures of *S. aureus* are kept even as low as 14°C until measurable growth has occurred in control tubes, a decrease in viable count is found in the ones containing penicillin. If the cultures were kept at 14°C for some time the killing action of the penicillin is observed.

The crux can readily be explained when it is considered that Hobby's work was done *in vitro*, while our set of experiments were carried out *in vivo*. The entire modern concept of combating infection is ultimately based on the physiological inactivation of pathogenic organisms, so that eventually the body defenses can attack and overcome the invading organism. The cooling of the infected area in the presented cases is not sufficient to stop entirely the bacterial division, and, therefore, some of the beneficial killing effects of penicillin noticed by Hobby *in vitro*, *must be in action*. Assuredly the bacteria are inhibited by the chilling effect, and as a result it seems probable that they are more susceptible to body defenses, namely phagocytosis and the formed antibodies. In this respect it was shown by Chandler, *et al* (6) that penicillin plus serum of persons who were infected with an organism was more effective in inhibiting the growth of that organism than penicillin plus serum of persons not infected.

As early as 1865 Cooke (7) recognized that ice checked the inflammation and suppuration of cancer which was secondarily infected. Smith (1) describes healing processes and production of new capillaries (where prolonged refrigeration has been employed in infected wounds) continued slowly, but satisfactorily in areas under constant refrigeration at 14°C.



There are additional possibilities of elucidating the reason for the excellent clinical results of the ice-penicillin therapy, namely, the growth-inhibiting properties of penicillin may be inactivated, or even destroyed, by substances of microbial origin. Abraham and Chain (8) found that *E. coli* contained an enzyme-like substance, penicillinase. Cavillito and Bailey (9) suggested that the main action of many antibiotic substances rests on their ability to interfere with the normal functions of sulfhydryl groups in bacterial metabolism. However, Frazier and Friden (10) suggest, "that the inhibition of antibiotics by sulfhydryl compounds may occur as the result of a chemical reaction involving the two substances and leading to the formation of an inactive product and does not require the hypothesis that the action of the inhibition is the restoration of normal equilibrium to an enzyme system involving SH compounds which produce a chemical reaction by which penicillin is inactivated", the ice may well slow up any chemical reaction which tends to destroy the activity of penicillin.

Gerber *et al* (11), suggested that when interrupted intragluteal penicillin is given, it is difficult to obtain a significant concentration of penicillin in many of the body tissues, and maintains that this is especially so in cases of subacute or chronic infections where the organisms are encased in dense granulomatous tissue. He also suggested injection of the penicillin directly into the infected site.

We have established that a significant level of penicillin may be maintained over a period of at least 31 hours at the site of a wound, when employing the ice-penicillin therapy.

Nine patients, with either a simple or severe wound of the lower extremity, were treated as follows:

Five ice bags were placed about the infected wound of the lower leg for one hour. The ice was removed and the debrided area about the wound was prepared with tincture of merthiolate. One hundred thousand units of penicillin dissolved in one per cent novocain were infiltrated about the wound. Two face towels were placed about the area to act as an aegis to the superficial tissues and the five ice bags were replaced. Samples of blood were withdrawn from the cephalic vein at frequent intervals. The first sample was taken six hours, and the last sample 31 hours after the injection. In all cases penicillin could be demonstrated in the blood stream at the end of 31 hours. As long as

a penicillin blood level can be demonstrated, the tissue at the site of injection must still contain a high level of penicillin, for Rantz and Kirby (12) showed penicillin to be rapidly excreted in the urine and found no evidence of destruction of penicillin in the tissues

When the ice-penicillin method of therapy is employed, not only is the penicillin kept in the local tissues for a long period of time, but the concentration of the penicillin at the site of infection must be much greater than that obtained when using the intragluteal method of administering the drug

### SUMMARY

1 Fifteen cases of infected varicose ulcers, forty-five cases of secondarily infected epidermophytosis, and sixty cases of wound infection were divided into groups and treated with either ice, interrupted intragluteal penicillin or ice, plus local infiltration of penicillin

2 The results of each type of therapy are compared in regards to the rapidity of disappearance of the signs and symptoms of infection, the total dosage of penicillin necessary to produce cure and the length of hospitalization

3 A discussion of the possible reasons for the excellent results of the ice-penicillin therapy is given

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# CIRCULATING ANTICOAGULANTS A TECHNIQUE FOR THEIR DETECTION AND CLINICAL STUDIES

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Several reports (1, 2, 3, 4, 5, 6, 7, 8, 9) have called attention to the fact that anticoagulant substances of unknown nature may appear spontaneously in the circulating blood, giving rise to hemorrhagic diathesis. Recently Allen and his co-workers observed the occurrence of a "heparin-like" substance in the blood of animals following exposure to ionizing radiation (10, 11) and in the blood of patients treated with nitrogen mustards (14). These investigators also suggest that in other conditions associated with thrombocytopenia there may be an increased amount of heparin-like substance in the blood (12).

No very satisfactory method for detecting circulating anticoagulant has been available. Techniques which have been employed include the determination of the effect on the clotting time of normal blood of admixture with a small portion of the whole blood (3) or decalcified plasma (1) to be tested. The use of blood or plasma to which an anticoagulant has been added is undesirable because of the likelihood that this extraneous material itself may influence the outcome of the test. On the other hand the use of untreated whole blood is technically difficult because of its inherent tendency to clot, except in those unusual instances in which the blood is spontaneously incoagulable. In order to overcome these difficulties we have used native (undecalcified) platelet-free plasma for our anticoagulant assays.

## I PREPARATION OF PLATELET-FREE PLASMA

Needles, syringes, test tubes and pipets to be used are treated with silicone<sup>1</sup> according to the technique described by Jaques and co-workers (11). A non-traumatic venipuncture is made with an 18 gauge silicone-treated needle. After several milliliters of blood have been with-

<sup>1</sup> General Electric Dri-Film 9987

drawn into a syringe, the needle is left in place, the syringe disconnected and its contents discarded. This serves to rinse the needle free of any tissue juice which may have entered it during the venipuncture. A clean silicone-treated syringe is then attached to the needle, and 10 to 20 milliliters of blood are carefully withdrawn. The blood is placed in a silicone-treated test tube in an icebath. Immediately thereafter the tube is centrifuged at about 7000 RPM (about 6000 g) at 4°C for 5 minutes to remove the blood cells and most of the platelets. The upper portion of the plasma is removed with a silicone-treated pipet and recentrifuged at about 12,000 to 14,000 RPM (about 17,500 to 22,000 g) for 10 minutes. The upper half of this plasma is then removed with a silicone-treated pipet and stored in a silicone-treated tube in an ice bath until required for use. Normal plasma obtained in this way may be kept for at least several hours at 4°C without spontaneous coagulation. Plasmas containing a minute trace of anticoagulant are spontaneously incoagulable even when transferred to glass tubes at 37°C.

## II ASSAY FOR ANTICOAGULANT

The presence of anticoagulant in the platelet-free plasma is demonstrated by observing its clot retarding effect on normal blood. Fifteen scrupulously clean Pyrex test tubes (13 mm x 100 mm) are mounted in a rack and to these are added physiological saline and platelet-free plasma as shown in Figure I.

With a silicone-treated needle and syringe sufficient blood is drawn from a normal individual to add one ml portions quickly to each of the 15 tubes. In order to equalize the time factor the front row of tubes is filled from left to right, the middle row from right to left, and the rear row from left to right as indicated by the directions of the arrows in the diagram. A stopwatch is started at the time of the venipuncture when the blood first appears in the syringe. After the tubes have been filled, they are shaken uniformly to mix their contents, and the rack is placed in a water-bath at 37°C. The tubes of the front row are tilted periodically until a solid clot forms, after which the tubes of the middle and finally the rear row are similarly observed. The clotting time of each tube is noted and recorded.

Significant prolongation of the clotting times in the tubes containing

the platelet-free plasma is indicative of the presence of anticoagulant. Repeated observations have shown that variations in the quantity of the saline used in the control tubes from 0 to 0.5 ml do not affect the clotting times. Normal platelet-free plasma may occasionally cause slight prolongation of the clotting time of normal blood. However, this rarely exceeds the clotting time of the saline controls by more than 7 minutes, whereas when anticoagulant is present the clotting time is usually greatly prolonged. When normal serum, fresh or stored, is

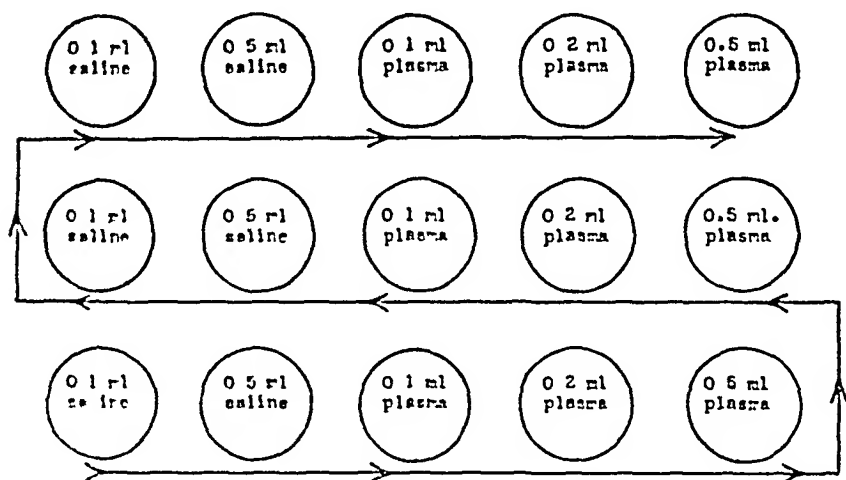


FIG. 1. Arrangement of tubes for anticoagulant assay. The control tubes contain saline and the remaining tubes platelet-free plasma in the quantities indicated. In performing the test, one ml portions of normal blood are added to each of the tubes in the order indicated by the arrows. The clotting time in each tube is recorded. The test is performed at 37°C.

used in place of the platelet-free plasma, there is no prolongation of the clotting time of normal blood. The circulating anticoagulants were always detectable by the use of platelet-free plasma, but in some patients not by the use of serum.

### III. APPRAISAL OF THE SENSITIVITY OF THE ASSAY

In order to determine the sensitivity of the assay procedure, tests on plasmas to which heparin<sup>2</sup> had been added *in vivo* and *in vitro* were undertaken. *In vitro* experiments were performed by the addition of

<sup>2</sup> Solution of Heparin Sodium salt, Lederle Laboratories, Inc.

heparin to normal platelet-free plasmas, and *in vivo* experiments by obtaining native platelet-free plasma from normal individuals before and after the intravenous injection of heparin. Tables 1 and 2 show the results of these experiments.

TABLE 1

*A comparative study of the results of anticoagulant assays before and after the in vitro addition of varying amounts of heparin to normal platelet-free plasma*

EXPERIMENT NUMBER	CONCENTRATION OF HEPARIN IN NORMAL PLATELET-FREE PLASMA	CLOTTING TIMES* OF 10 ML PORTIONS OF NORMAL BLOOD CONTAINING				CLOTTING TIME OF PLATELET FREE PLASMA IN GLASS TUBES
		Saline 0.1 to 0.5 ml	0.1 ml platelet free plasma	0.2 ml platelet free plasma	0.5 ml platelet free plasma	
	<i>mgm per ml</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>
I	None		19	19	20	20
	0.01	19	35	42	>480	No clots
II	None		21	24	20	11
	0.01	22	31	39	>128	No clots
III	None		20	19	17	11
	0.001	19	24	31	42	No clots
IV	None		14	—	12	11
	0.0005	15	21	23	26	No clots
V	None		20	21	20	—
	0.0005	21	20	22	29	—
VI	None		18	19	18	—
	0.00025	15	22	22	29	—

\* Clotting times were performed by a modified Lee-White method using 3 tubes at 37°C. Only the clotting time of the third tube is recorded in the tables for the sake of brevity.

From the results in Table 1 it is apparent that with plasma heparin concentrations of 0.001 mgm per ml and greater, this assay gives unequivocally positive results. Even with plasma heparin concentrations as low as 0.00025 mgm per ml there is a definite prolongation of the clotting time of normal blood, although this is within the limits sometimes seen with normal plasmas. Table 2 shows that following the intravenous injection of amounts of heparin too small to affect

TABLE 2

*Comparative Study of the Effects of Anticoagulant Assays on Normal Individuals Before and After Intravenous Injections of Varying Amounts of Heparin*

EXPERIMENTAL GROUP		CLOTTING TIMES OF 1.0 ML. PORTIONS OF NORMAL FLOOD CO. TAT 1 C						CLOTTING TIME OF PLATELET FREE PLASMA IN GLASS TUBES
		WT. OF FLOOD CO. TAT 1 C	Saline (0.1 to 0.5 ml.)	0.1 ml. of platelet free plasma	0.2 ml. of platelet free plasma	0.5 ml. of platelet free plasma	0.5 ml. of platelet free plasma + 0.1 ml. of toluidine blue*	
		min	min	min	min	min	min	min
I	Before heparin	19		27	—	27	—	26
	16 minutes after 10 mgm. of heparin intravenously	31	20	39	43	61	27	No clots
II	Before heparin	20		24	25	23	—	54
	12 minutes after 7.5 mgm. of heparin intravenously	19	22	23	25	33	28	No clots
III	Before heparin	17		13	15	15	—	42
	21 minutes after 5 mgm. of heparin intravenously	18	14	19	20	20	23	No clots
IV	Before heparin	17		22	24	26	—	47
	16 minutes after 5 mgm. of heparin intravenously	24	22	26	28	30	35†	No clots

\*Toluidine blue, Special Patch # 2, Serial E 1204, Abbott Laboratories. As a screening test a concentration (0.5%) was used. One tenth milliliter of this solution was added to 1.0 ml. of normal blood containing 0.01 mgm. of heparin per milliliter. Control values are the clotting time to normal.





## Hemorrhagic diathesis with thrombocytopenia

9	Acute leukemia (Infant After 6.5 mgms aminopterin)	36 hrs	—	23	28	23	28	—	No clots	Platelets 18,000 1' Positive tourniquet test
10	Acute leukemia (Before aminopterin) (After 20.0 mgm aminopterin)	19 9	27 15	17 18	24 21	24 21	24 19	23 17	27 11	Platelets 44,000 11' Negative tourniquet test Platelets 22,000
11	Acute leukemia (After 5.0 mgm aminopterin)	88	46	16	18	26	22	26	No clots	Platelets 12,000
12	Acute leukemia	7	18	18 12	19 11	— 14	18 12	21 14	— 18	Platelets 26,000 16' Negative tourniquet test Bleeding time
13	Chronic lymphatic leukemia	27	—	17	—	17	18	27	180	Platelets 22,000 11' Positive tourniquet test Bleeding time
14	Thrombocytopenic purpura, primary	15	25	15	16	16	15	21	27	Platelets 30,000 10' Positive tourniquet test Bleeding time
15	Thrombocytopenic purpura, primary	8	21	15	14	14	15	14	15	Platelets 40,000 10' Positive tourniquet test Bleeding time
16	Refractory anemia with thrombocytopenia	25	38	20	19	19	19	19	90	Platelets 90,000 90' Positive tourniquet test Bleeding time
*17	B. welchii septicemia	52	—	18	27	28	32	30	No clots	Platelets 70,000 15' Positive tourniquet test Bleeding time
18	Marchiafava-Micheli syndrome	29	—	16	—	—	12	12	No clots	Platelets 68,000 1' Positive tourniquet test Bleeding time

## Hemorrhagic diathesis without thrombocytopenia

19	Non-thrombocytopenic purpura	14	—	22	21	24	20	21	11	Platelets & bleeding time normal Positive tourniquet test
20	Atypical hemorrhagic diathesis	35	—	22	18	18	18	—	No clots	Platelets, bleeding time, and tourniquet test normal
21	Multiple myeloma	43	—	11	11	11	11	—	24	Platelets & bleeding time normal Positive tourniquet test

TABLE 3—Continued

SUB- JECT	DIAGNOSIS	SUBJECT'S WHOLE BLOOD CLOTTING TIME WITH ADDED		ANTICOAGULANT ASSAY										CLOTTING TIME OF PLATELET- FREE PLASMA IN GLASS TUBES AT 37°C	REMARKS
				Clotting time of 10 ml portions of normal blood containing											
		0.1 ml of saline		0.1 ml of toluidine blue (0.5%)		Subjects platelet free plasma as follows									
						Saline (0.1 to 0.5 ml)		0.1 ml		0.2 ml		0.5 ml			
Hemorrhagic diathesis with advanced liver disease															
*22	Fatal acute hepatitis	109	—	min	min	21	21	21	31	—	min	No clots	Prothrombin 10% of normal Antithrombin anticoagulant		
*23	Carcinoma of breast with carcinomatosis of liver	>24 hrs	>24 hrs			17	32	37	>14 hrs	—		No clots	Prothrombin 5% of normal Antithrombin anticoagulant		
†24	Fatal acute hepatitis	22	—	—	min	17	18	17	19	17		No clots	Antithrombin anticoagulant Prothrombin <5% of normal		
Hypoprothrombinemia due to dicumarol															
*25	Coronary occlusion	41	—	—	min	20	20	21	26	—	min	No clots	Prothrombin 10% of normal		
		26	26	26	min	19	22	26	34	—	min	No clots	Prothrombin 10% of normal		
		18	—	—	min	16	20	20	18	18		35	Prothrombin 20% of normal		
*26	Fatal facial cellulitis	55	—	—	min	22	28	29	32	22		No clots	Prothrombin 30% of normal		
		15	28	28	min	18	20	20	25	18		No clots	Prothrombin 40% of normal		
27	Coronary occlusion	55	40	40	min	18	20	23	24	20		No clots	Prothrombin <5% of normal		

Hodgkins disease treated with nitrogen mustards (No hemorrhagic diathesis)

		22	—	16	17	17	20	18	—	
28	Hodgkins disease	11	18	20	20	20	23	19	16	Before nitrogen mustards
		18	—	19	19	17	19	20	18	Three days after treatment
		19	—	17	23	21	19	18	19	Eleven days after treatment
29	Hodgkins disease	16	—	15	16	18	18	18	52	Four weeks after treatment
		27	—	16	17	17	14	19	37	Before nitrogen mustards
		26	—	20	17	18	21	18	180	Eight days after treatment
30	Hodgkins disease	14	17	13	13	16	8	27	25	Two weeks after treatment
		20	20	14	16	13	19	14	54	During treatment
		31	—	17	—	21	24	22	180	One week after treatment
										Two weeks after treatment

Subjects without evidence of hemorrhagic diathesis

		23	—	19	19	19	20	—	20	
31	Diabetes mellitus	32	—	21	23	—	28	—	140	—
32	Diabetes mellitus			21	20	21	20	—	—	—
				15	18	19	18	—	—	—
33	Post-op appendectomy	39	—	18	23	22	22	—	45	—
34	Spastic clubfoot	19	—	20	27	—	27	—	26	—
35	Carcinoma of cervix	16	20	18	20	—	20	—	18	Irradiation sickness, moderate
36	Normal	17	—	22	22	24	26	—	47	—
37	Normal	22	—	16	18	18	18	21	39	—
38	Normal	16	—	16	16	18	18	17	26	—
39	Normal	17	—	14	13	15	15	—	42	—
40	Normal	15	—	19	17	19	19	—	17	—
41	Normal	20	—	22	24	25	23	—	19	—

\* Positive anticoagulant assay

† This patient did not suffer from hemorrhagic diathesis, but is included for comparison with other patients suffering from liver disease

appreciably the venous clotting time, this assay will still give suggestively positive results

#### IV CLINICAL STUDIES

Forty-one subjects with or without evidence of hemorrhagic diathesis were studied using the technique described. These studies are summarized in Table 3

Of the 41 subjects studied, eight showed evidence of circulating anticoagulants. In only one (#26) of these eight was the test reverted to normal by the addition of toluidine blue *in vitro*, suggesting the presence of a heparin-like substance. This patient had a severe facial cellulitis and hypoprothrombinemia due to dicumarol. In another patient with hypoprothrombinemia due to dicumarol (#25), the anticoagulant assay was positive on one occasion. Both of these patients had negative assays on subsequent tests. Of the six hemophilic patients studied, only one (#1) was found to have a circulating anticoagulant. Two patients (#7 and #8) previously reported (9) as having an undiagnosed disease with circulating anticoagulant showed markedly positive tests by our method. A positive test occurred in a patient (#17) with a bacillus welchii septicemia and thrombocytopenia following a septic abortion. In two of the three patients (#22, #23, #24) dying with severe liver disease, the anticoagulant assays were positive. We believe that these two patients (#23, #24) had antithrombic anticoagulants, because the clotting times of their plasmas on the addition of thrombin solution was prolonged markedly as compared to the clotting times of normal plasmas similarly treated.

In nine cases of thrombocytopenia (#9, 10, 11, 12, 13, 14, 15, 16, 18) the anticoagulant assays were negative. Three patients with leukemia (#9, 10, 11) showed negative assays after aminopterin therapy. Three patients with Hodgkins disease (#28, 29, 30) followed from two to four weeks after treatment with nitrogen-mustard<sup>3</sup> have had negative assays. Other subjects with and without hemorrhagic diathesis showed negative assays as listed in Table 3

#### DISCUSSION

A sensitive method for the detection of circulating anticoagulant by the use of native platelet-free plasma has been presented. In

<sup>3</sup> Methyl-bis (B-chloroethyl) amine hydrochloride (Merck), 0.1 mgm per kilo body weight on 4 consecutive days

every instance in which circulating anticoagulant was found, the platelet-free plasma was spontaneously incoagulable. This was true even when the clotting time of the whole blood was not markedly prolonged.

In controlled experiments with the addition *in vivo* and *in vitro* of amounts of heparin too small to affect greatly the whole blood clotting times, the detection of anticoagulant was nevertheless quite easy. It seems reasonable to assume that in any instance in which the whole blood clotting time is definitely prolonged due to the presence of anticoagulant, this assay would be unequivocally positive. Allen and his co-workers (12) suggest that a heparin-like anticoagulant may be associated with the hemorrhagic diathesis of certain cases of severe thrombocytopenia. In our small series of such cases we have been unable to detect the presence of any circulating anticoagulant even when the patients' whole blood clotting times were markedly prolonged and the patients were suffering from severe bleeding. If circulating anticoagulants occur in such patients as reported by Allen and his co-workers (12), their occurrence appears not to be a regular phenomenon.

In our experience, normal platelet-free plasma invariably clots in a relatively short time when placed in glass tubes at 37°C. This spontaneous coagulation of normal platelet-free plasma is completely inhibited by the addition of very minute amounts of heparin in concentrations too small to delay the clotting of whole blood. It seems quite reasonable to assume that the increased concentration of heparin required to inhibit or delay the coagulation of whole blood is roughly related to the number of platelets present. Allen and his associates (12) have described a test in which they determine the amount of protamine required to inactivate a given amount of heparin added *in vitro* to whole blood. These authors found that more protamine was necessary to restore the normal clotting time in thrombocytopenic blood than was required by normal controls, and suggested that in thrombocytopenia an increased amount of a heparin-like substance may be present in the blood. In view of our studies, it is quite apparent that the results of their tests would be influenced markedly by the platelet concentration. It seems to us that the increased susceptibility of thrombocytopenic blood to heparin, as reported by these authors, can be adequately explained by the deficiency of platelets without assuming the existence of an increased amount of a heparin-like substance circulating in the blood.

It can be seen from examination of Experiment III in Table 2 that, with a very low concentration of heparin, toluidine blue in the concentration used (0.5%) did not restore the clotting time of heparinized plasma to normal. This serves to reemphasize the well known fact that heparin inhibitors such as toluidine blue and protamine are in themselves anticoagulants. These substances correct a delayed clotting time due to heparin only when they are provided in concentrations just sufficient to inactivate the amount of heparin present. Further evidence of the anticoagulant action of these heparin inhibitors when present in excess is presented by the frequent prolongations of the clotting times when toluidine blue was added *in vitro* as shown in Table 3. Because of the reported value of the administration of anti-heparin compounds in hemorrhagic conditions associated with thrombocytopenia (12), two patients suffering from acute leukemia were given toluidine blue and protamine<sup>4</sup> intravenously in recommended doses. These substances failed to affect the hemorrhagic manifestations in any way. Furthermore, following the intravenous injection of from 50 to 200 mgm of protamine the venous clotting times were greatly prolonged. In view of the anticoagulant effects of these drugs it appears quite possible that they may aggravate hemorrhage when given to patients who do not have hyperheparinemia.

It was not possible to ascertain the exact nature of the circulating anticoagulants in those cases in which they were found. Detailed studies on the anticoagulants of three patients (#1, 7, 8) have already been reported (9). One patient (#26) who had a severe facial cellulitis and hypoprothrombinemia due to dicumarol, showed the possible presence of a heparin-like substance on one occasion, but repeated studies did not confirm this, and we were unable to draw definite conclusions as to the nature of the anticoagulant. The nature of the anticoagulant in the patient (#17) with bacillus welchii septicemia following a septic abortion was not determined because the patient died before further studies could be carried out. One patient (#25) with hypoprothrombinemia due to dicumarol given therapeutically for a coronary occlusion failed to show the presence of the anticoagulant after dicumarol was discontinued and the prothrombin rose.

The patients with liver disease were of great interest. These three

<sup>4</sup> Protamine, Eli Lilly and Company

patients (#22, 23, 24) were severely ill with ultimately fatal liver damage. The two (#22, 23) with positive anticoagulant assays had strong antithrombic anticoagulants in addition to a severe hypoprothrombinemia. As early as 1913, Whipple (15) noted that the plasma of a severely jaundiced patient with liver damage delayed the recalcified clotting time of normal dog plasma. The existence of increased *serum* antithrombic activity as an etiological factor in the hemorrhagic diathesis in obstructive jaundice has been postulated by other investigators (16, 17). However, their work is now subject to a more critical review in the light of more recent investigations on the hypoprothrombinemia associated with liver disease. Dyckerhoff and Marx (18) have presented experimental evidence for their opinion that an increase in *serum* antithrombin as well as hypoprothrombinemia may be concerned in the hemorrhagic diathesis in rabbits following ligation of the common bile duct. These authors stated that the question as to whether this was due to an increase in the normal *serum* antithrombin or to a specific inhibitory substance must be left to further investigation.

We are of the opinion that the antithrombins in our two cases were of a different nature than normal serum antithrombin in that they were truly anticoagulant. Clotting of these patients' *plasmas* by the addition of thrombin solution was markedly prolonged as compared to the clotting of normal plasmas. This was true even though the plasma fibrinogen levels were adequate. Therefore, we believe that these patients had active antithrombic anticoagulants in their plasmas rather than merely an increased thrombin-inactivating potency of their sera. Detailed studies on these and other cases of jaundice will be reported later.

In our experience, platelet-free plasmas from normal individuals invariably clot when placed in glass tubes at 37°C. If the platelet-free plasma clots, one can be certain that there is no anticoagulant present. When anticoagulant is demonstrated, its potency may be estimated by determining the smallest amount of platelet-free plasma necessary to prolong the clotting time of normal blood. One of our patients (#7) had an anticoagulant of such potency that 0.005 ml. of his platelet-free plasma produced an appreciable prolongation of the clotting time of 1 ml. of normal blood.



## SUMMARY

1 A sensitive method for the detection of circulating anticoagulants is described. This method involves the use of platelet-free plasma to which no anticoagulant has been added *in vitro*.

2 The assay was found to be sensitive for concentrations of heparin too low to affect the whole blood clotting times appreciably.

3 Results obtained in 41 human subjects are presented and discussed.

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# PHYSIOLOGICAL STUDIES IN CONGENITAL HEART DISEASE\*

## VIII THE PHYSIOLOGICAL FINDINGS IN TWO PATIENTS WITH COMPLETE TRANSPOSITION OF THE GREAT VESSELS

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Previous papers from this laboratory have presented the results of physiological studies in patients with various congenital cardiac malformations. It is the purpose of this communication to report the results of physiological studies in two cases of complete transposition of the great vessels. In this congenital anomaly the aorta arises from the right ventricle, the pulmonary artery from the left. The great veins enter the heart normally (Fig 1). The cases reported here were selected out of a series of five, since the results of the physiological studies could be correlated with clinical and postmortem findings.

### METHODS

The methods used in these studies have been described in detail in the first paper of this series (1). The Fick principle has been applied to determine the blood flow through various parts of the circulation. A definition of terms and a statement of the formulae as they apply to transposition of the great vessels follow.

Systemic flow is the volume of blood passing through the peripheral arterial vessels per minute.

$$a \text{ Systemic flow (ml per min)} = \frac{\text{Oxygen uptake (ml per min} \times 100)}{\left( \begin{array}{c} \text{O}_2 \text{ content of} \\ \text{peripheral ar-} \\ \text{terial blood} \\ \text{(vol \%)} \end{array} \right) - \left( \begin{array}{c} \text{O}_2 \text{ content of} \\ \text{mixed venous} \\ \text{blood} \\ \text{(vol \%)} \end{array} \right)}$$

Pulmonary artery blood flow is the blood flowing through the pulmonary valve into the pulmonary artery per minute.

\* This study was supported by a grant from the Commonwealth Fund and the Carolyn Rose Strauss Foundation.

b Pulmonary artery flow (ml per min)

$$= \frac{\text{Oxygen uptake (ml per min} \times 100)}{\left( \begin{array}{c} \text{O}_2 \text{ content of} \\ \text{pul vein blood} \\ \text{(vol \%)} \end{array} \right) - \left( \begin{array}{c} \text{O}_2 \text{ content of} \\ \text{pul art blood} \\ \text{(vol \%)} \end{array} \right)}$$

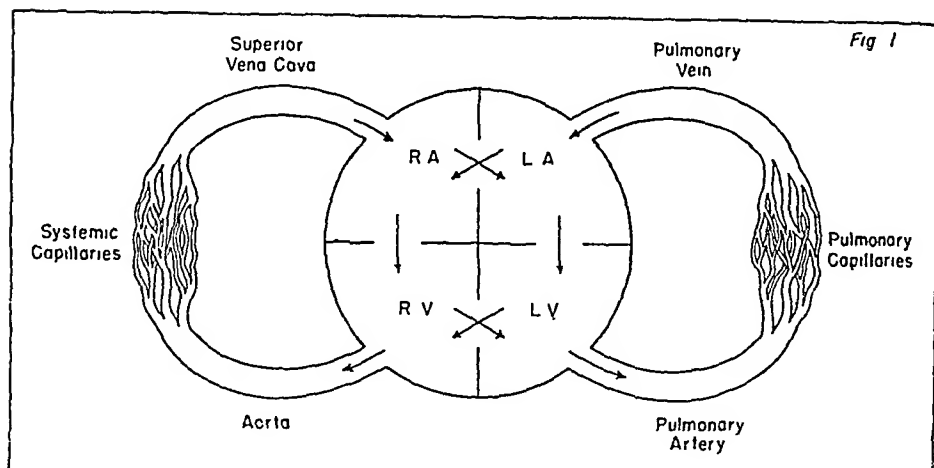


FIG 1 Illustrates the anatomical arrangement present in congenital transposition of the great vessels. Auricular and ventricular septal defects have been included in this diagram to demonstrate possible means for admixture of blood between the two circulations.

The effective pulmonary blood flow is the volume of mixed venous blood which, after returning to the right auricle, eventually reaches the pulmonary capillaries.

c Effective pulmonary blood flow = 
$$\frac{\text{Oxygen uptake (ml per min} \times 100)}{\left( \begin{array}{c} \text{O}_2 \text{ content of} \\ \text{pul vein blood} \\ \text{(vol \%)} \end{array} \right) - \left( \begin{array}{c} \text{O}_2 \text{ content of} \\ \text{mixed venous} \\ \text{blood (vol \%)} \end{array} \right)}$$

In calculating these flows, certain assumptions are made. When the oxygen content of pulmonary vein blood cannot be directly determined, a figure based on the assumption that pulmonary vein blood is 95 percent oxygenated is substituted in formulae b and c. In the presence of a ventricular septal defect, the oxygen content of right auricular blood is used to represent mixed venous blood in the determination of the systemic and the effective pulmonary blood flows. In

the presence of an auricular septal defect, however, the oxygen content of vena caval blood is assumed to be representative of mixed venous blood

Blood oxygen values were determined from samples obtained during cardiac catheterization and by arterial puncture. Analysis of respiratory gases was carried out according to methods previously described (2)

*Case 1 J C B (Male, age 7, date of study, December 7, 1947)*

The child was born at term following a normal pregnancy. At the age of two weeks when, because of persistent vomiting, he was taken to a pediatrician, a diagnosis was made of congenital heart disease with cyanosis. Although his development was slow and his exercise tolerance definitely limited, by the age of six years he was able to walk four or five blocks. During his first four years he always slept in the knee-chest position and often squatted to relieve dyspnea after exercise.

At the age of four months, the patient had bloody diarrhea, he also had some hematuria during his illness with chickenpox, and in the two years preceding admission he had had frequent episodes of "crops of petechiae" which disappeared spontaneously without specific therapy.

Physical examination revealed a small, cyanotic white boy of seven. He had clubbing of the fingers and toes. Tiny telangiectasis were present over the face, the volar surfaces of the forearms, and the legs. Capillary fragility tests were negative.

The pulse rate was 120 with regular rhythm. The blood pressure in the arms was 110/80. The heart percussed 6.5 cm. to the right of the midsternal line in the 5th interspace. A systolic thrill and murmur were most marked in the 2nd interspace to the left of the sternum, and the murmur could be heard over the left chest both anteriorly and posteriorly.

The lungs were clear to percussion and auscultation. There was no peripheral edema, and the liver and spleen were not enlarged. The remainder of the physical examination disclosed nothing remarkable.

The electrocardiogram showed right axis deviation and right ventricular hypertrophy. Routine laboratory studies revealed a hematocrit of 77.5 (Win-trobe), a hemoglobin of 23.5 grams (Sahli), and a red blood cell count of 9.5 million. On fluoroscopy the heart was slightly enlarged to the right, there was some evidence of a pulmonary conus, and the hilar shadows were thought to be too prominent for a typical tetralogy of Fallot. There was a left aortic arch. The pulmonary "window" was not clear.

Angiocardiography was performed with the following impression: "1. Complete transposition of the great vessels with the aorta arising entirely from the right ventricle. 2. Interauricular septal defect with the main overall shunt taking place from the right to the left auricle with re-opacification of the right ventricle."

and aorta The route of the venous blood to the lungs cannot be clearly ascertained "

*Physiological studies* The standard exercise test was performed twice On the first occasion the oxygen consumed per liter of ventilation rose from 27.0 cc to 31.0 cc A second test showed a fall from 34.2 cc to 28.0 cc Pertinent data obtained during cardiac catheterization are shown in Fig 2 Significant findings include

- 1 All four chambers of the heart were catheterized

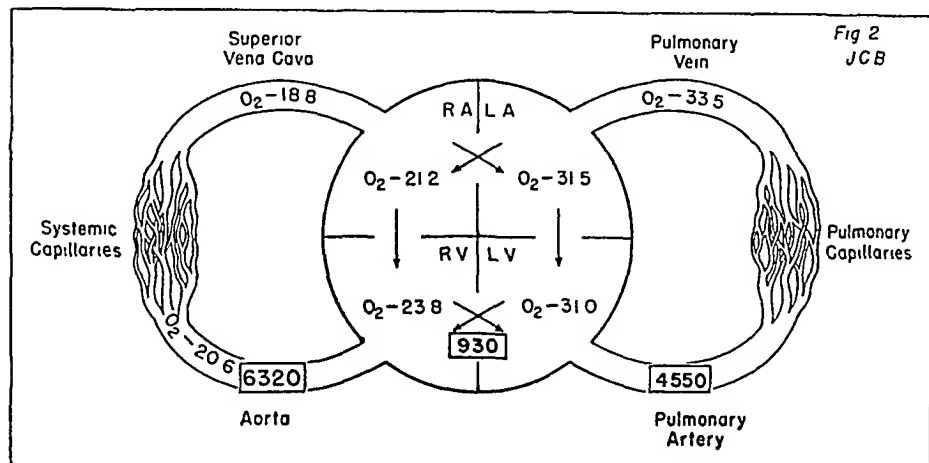


FIG 2 Shows the findings in a patient (J C B) with transposition of the great vessels The increase in blood oxygen content from superior vena cava to right auricle demonstrates the presence of an auricular septal defect The presence of a ventricular septal defect is shown by a rise in blood oxygen content from right auricle to right ventricle

- 2 The oxygen content of the right auricular blood exceeded that of the superior vena cava by 2.4 volumes percent The oxygen content of the right ventricular blood was 2.6 volumes percent higher than that of the right auricular blood

- 3 The oxygen content of left auricular and left ventricular blood were within 0.5 volumes percent of one another The oxygen saturation in these two chambers was about 93 percent

- 4 The oxygen saturation of peripheral arterial blood was 59 percent

- 5 Right ventricular and left ventricular pressures were 41/27 mm Hg and 39/26 mm Hg respectively

*Comments* The presence of both auricular and ventricular septal defects is suggested by the fact that all four chambers of the heart were intubated. Further evidence of the presence of these defects is that the oxygen content of right auricular blood is higher than that of the superior vena cava, and the oxygen content of right ventricular blood exceeds that of the right auricle. The oxygen content of peripheral arterial blood is lower than that of right ventricular blood. This may be due to the fact that the ventricular intracardiac samples were taken when the catheter tip lay close to the septal defect. The effective pulmonary flow was 930 cc/min/M<sup>2</sup>.

On the 18th of December, 1947, a right thoracotomy was performed and the diagnosis of transposition of the great vessels was confirmed. The origin of the pulmonary artery was posterior to the aorta. The right pulmonary artery was divided and its distal end was anastomosed to the end of the right subclavian artery. An anastomosis between the proximal end of the right pulmonary artery and the side of the superior vena cava was performed. At the time of the operation the mean pulmonary artery pressure was 48 mm Hg.

Immediately following the operation the patient's color seemed improved and the peripheral arterial oxygen saturation was 82 percent. He was digitalized with lanatoside c and was maintained on digifolin. About 12 hours postoperatively, however, the child began to respire with increasing difficulty, cyanosis developed and he became unconscious. Artificial respiration was of no avail.

Autopsy revealed the following pertinent findings: complete transposition of the great vessels, ventricular septal defect, patent foramen ovale, hypertrophy of the right and left ventricles.

The heart weighed 260 grams. Both ventricles were enlarged, especially the right. The right auricle was dilated and received the veins normally. The foramen ovale was covered by a membrane in which there were several openings. There was a high ventricular septal defect which was 8 mm in diameter and partly covered by endocardium. The aorta arose from the right ventricle and its valve was normal. The left auricle was slightly dilated. The mitral valve was normal. The pulmonary artery arose from the left ventricle and coursed behind the ascending aorta. The pulmonic valve was normal, but the main trunk of the vessel was dilated and bulged at the point of division. The ductus arteriosus was closed.

*Case 2* L. B. (Male, age 5 years, date of study, March 28, 1948)

The child was delivered at term after a normal pregnancy. He was cyanotic at birth and remained so. His development was moderately retarded and his exercise tolerance was markedly limited. He had had no episodes suggesting cardiac failure.

Physical examination disclosed a slight but fairly well nourished child of 5. The lips and mucous membranes were markedly cyanotic and the skin had a dusky

hue The fingers and toes were clubbed and the nail beds were cyanotic The left portion of the thoracic cage was larger in both diameters than the right The lungs were clear to percussion and auscultation The blood pressure in the arms was 90/65 mm Hg The left heart border was percussed 7.5 cm from the mid-sternal line in the 5th interspace The heart sounds were clearly heard, the rate and rhythm were normal, and no murmurs were heard The liver was palpated 3 cm below the costal margin and the spleen was felt

Fluoroscopy showed the heart to be moderately enlarged in both the transverse and anteroposterior diameters The pulmonary conus was slightly prominent The markings at the right hilum were increased and pulsations were observed in the hilar vessels A left aortic arch was demonstrated There was no enlargement of the left auricle and both ventricles appeared prominent in the left anterior oblique view

Electrocardiographic findings showed a right axis deviation with evidence in the unipolar precordial leads of right ventricular hypertrophy The hematocrit was 97 (Wintrobe), the hemoglobin was 18.8 grams (Sahl), and the red cell count was 8.71 million

Angiocardiography demonstrated passage of the contrast medium into both ventricles and filling of the aorta within two seconds after injection Visualization of the pulmonary tree occurred later The diagnosis was transposition of the great vessels with auricular septal defect

*Physiological studies* Pertinent data obtained from cardiac catheterization are shown in Fig. 3 The significant findings are as follows

- 1 The oxygen content of the right ventricular blood was 2.7 volumes percent higher than that of the right auricular blood

- 2 The aorta was catheterized through the right ventricle, and the oxygen content of aortic blood was 3.1 volumes percent higher than that of right ventricular blood

- 3 The pressure in the right ventricle was 42/25 mm Hg A dampened pressure, 62/55, was recorded from the aorta

*Comments* The rise in oxygen content of the blood from the right auricle to the right ventricle indicates the presence of a ventricular septal defect allowing left to right admixture of blood The observation that the oxygen content of the aortic blood is higher than that of right ventricular blood indicates the presence of a patent ductus arteriosus, shunting blood from the pulmonary artery into the aorta The oxygen difference between the right ventricular and aortic blood also may be the result of the ventricular septal defect, through which a large volume of oxygenated blood is diverted into the right ventricle

The pulmonary artery flow cannot be calculated since it was not possible to catheterize either the pulmonary artery or the left ventricle. The effective pulmonary flow was 500 cc/min/M<sup>2</sup>.

On the 20th of April, 1948, a right thoracotomy was performed. The diagnosis of transposition of the great vessels was confirmed. The pulmonary artery was lying posterior to the aorta. The right pulmonary artery was divided and an anastomosis between the proximal end and the side of the superior vena cava was

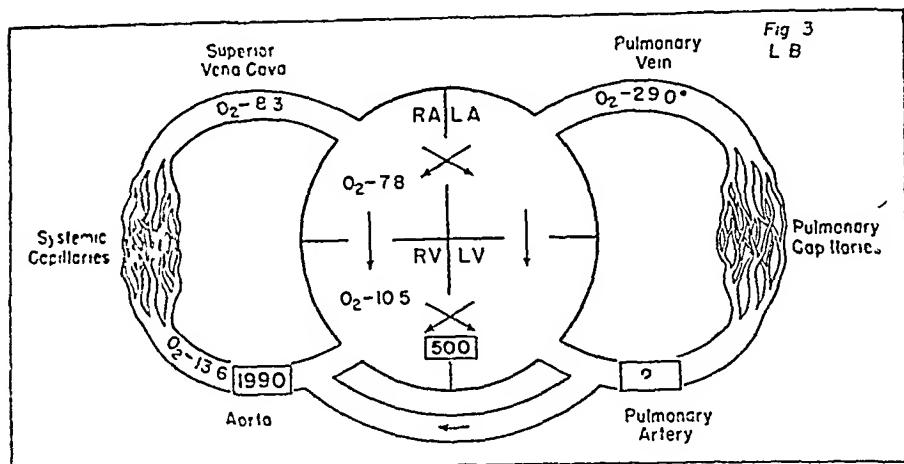


FIG 3 Is a diagrammatic representation of the findings in a patient with transposition (L B). Although the blood oxygen contents of the superior vena cava and right auricle are essentially in agreement, post-mortem examination revealed a septal defect (patent foramen ovale). The rise in blood oxygen content from right auricle to right ventricle discloses the presence of a ventricular septal defect. The increased O<sub>2</sub> content of aortic blood compared to that of right ventricular blood suggests the presence of a patent ductus arteriosus. Shunt of blood in one direction through these defects must be compensated by concomitant admixture in the opposite direction.

performed. The distal end was ligated. Shortly after the chest wall was closed, respirations became poor and finally ceased.

Autopsy revealed the following findings: Transposition of the aorta and the pulmonary artery, patent foramen ovale, patent ductus arteriosus, ventricular septal defect, hypertrophy and dilatation of both ventricles, dilatation of the pulmonary artery and its branches, and arteriosclerosis of the pulmonary artery. The pulmonary artery was larger in circumference than the aorta. There was a high ventricular septal defect measuring 1.2 by 1 cm. The cusps of the mitral, tricuspid and aortic valves were normal, the foramen ovale and ductus arteriosus were both patent.



## DISCUSSION

The results reported in this paper demonstrate the difficulties encountered in the diagnosis of transposition of the great vessels by physiological methods alone

The exercise test if of no particular value in the recognition of transposition A fall in the rate of oxygen consumed per liter of ventilation from rest to exercise has been observed in the two cases reported in this paper A decline in this ratio, however, also occurs in individuals with pulmonic stenosis

Catheterization of the aorta through the right ventricle accomplished in patient L B is suggestive, but not definitive evidence, since the aorta is not infrequently intubated in tetralogy of Fallot through a high septal defect

Recognition of this malformation by physiological tests is further hindered by difficulties encountered in the estimation of the pulmonary artery flow Without advance knowledge of the abnormal origin of the great vessels, the blood  $O_2$  content of right, rather than left ventricle, is erroneously used for the calculation of the pulmonary blood flow Even if the correct diagnosis is suspected, calculation of the pulmonary artery flow is difficult since sampling of left ventricular blood is not easily accomplished Once the diagnosis of transposition of the great vessels has been suggested by fluoroscopy or angiocardiography, however, physiological methods are of value in elucidating the intracardiac hemodynamics

Some means must exist whereby oxygenated blood can reach the systemic circulation if transposition of the great vessels is to be compatible with extra-uterine life Such interchange can take place through a patent ductus arteriosus, a patent foramen ovale, or a septal defect In the first case described in this report (J C B) both a patent foramen ovale and a ventricular septal defect were present In the second case (L B) a patent ductus arteriosus, a patent foramen ovale, and a ventricular septal defect were found

In cases of septal defect without transposition, the overall intracardiac shunt is unidirectional and may be computed as the difference between the volume of the systemic and the pulmonary flows Thus, when the systemic blood flow exceeds that through the pulmonary artery, the overall direction of the shunt is from right to left

Conversely, if the pulmonary artery flow exceeds the systemic flow, the overall direction of the shunt is from left to right. Equilibrium of the blood volumes between the pulmonary and the systemic circulation is maintained in these cases since the quantity of the overall shunt is returned to the side of the heart from which it was originally diverted.

On the other hand, in transposition of the great vessels, a unidirectional shunt cannot exist continuously as it would lead to progressive depletion of the circulating blood volume in either the pulmonary or the systemic circulation. The crucial hemodynamic alteration in transposition of the great vessels is that the volume of blood, shunted from pulmonary to systemic circulation or vice versa, cannot return to that side of the heart from which it was diverted unless there is an equivalent, opposite shunt. One must postulate, therefore, that in this condition equal volumes of blood are reciprocally shunted from one circulation to the other.

Since the effective pulmonary blood flow is the volume of venous blood which, after its return to the right auricle, is eventually aerated in the lung, it must be represented by the right to the left shunt. According to the considerations presented above, an equivalent amount of blood also must be shunted from left to right. Thus, a computation of the effective flow by methods outlined above also reveals the amount of right to left shunt and the corresponding left to right shunt.

Although, eventually, the shunt must be equal in both directions, the possibility exists that over a short period of time the shunt may be predominantly toward one side. Several observations bear out this possibility. First, it has been shown that the peripheral oxygen saturation is a direct function of the ratio of the effective pulmonary blood flow to the systemic circulation (2). However, in the first case (J C B) the effective pulmonary flow represents 14 percent of the systemic circulation, and the peripheral arterial oxygen saturation is 59 percent. In the second case (L B), the effective flow is 25 percent of the systemic flow, but the peripheral arterial saturation is only 44 percent. Since mixed venous and peripheral arterial blood samples were not collected simultaneously, a change in the direction of the intracardiac shunt between sampling might explain this discrepancy. A second observation which may be construed as evidence for the possibility that the direction of the intracardiac shunt may shift periodically

is the fluoroscopic finding of Taussig that the auricles may show a rhythmic increase and decrease in size, independent of respiratory changes (3) It becomes apparent, therefore, that measurement of the effective flow, or in other words, the right to left shunt, is valid only for a given time

It might be expected that intracardiac pressures might reflect these changes in volume or direction of the intracardiac shunt Pressures obtained in the first patient (J C B) were 41/27 from the right ventricle and 39/26 from the left, and the contours of the pressure curve were similar No variations in amplitude or contour occurred which could be interpreted as significant Adequate pressure tracings were recorded from the right auricle Again, no intermittent change was noted Consequently, while the possibility exists that the volume and direction of the intracardiac shunt may vary over short periods of time, no definite physiological evidence for this phenomenon has been observed

#### SUMMARY

Physiological studies have been performed on two patients with complete transposition of the great vessels The results obtained were correlated with the clinical and postmortem findings

A discussion of the intracardiac hemodynamics has been presented

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# MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

HURD HALL, JOHNS HOPKINS HOSPITAL

MONDAY, DECEMBER 13, 1948—8 15 P M

*Dr Harvey* The first paper on the program this evening is on "The Clinical Studies of Two Polymethylene "

I am very sorry to say that in printing up the program the name of one of the collaborators was inadvertently omitted—the name of Dr Holaday of the Department of Pharmacology

The paper will be given by Dr Grob

*Some Preliminary Observations on The Neuromuscular and Ganglionic Blocking Action in Man of bis-Trimethylammonium Decane and Pentane Diiodide* DAVID GROB, A McGEHEE HARVEY AND DUNCAN A HOLADAY

d-Tubocurarine chloride blocks conduction across the neuromuscular junction, and its ability to thereby produce relaxation of skeletal muscle has been employed during surgical anesthesia and convulsive shock therapy Tetraethylammonium chloride blocks conduction across autonomic ganglia, and has been employed to produce vasodilatation in patients with peripheral vascular disease, and in the study of the various types of hypertension Both of these compounds have some undesirable features d-Tubocurarine, when administered in amounts sufficient to produce relaxation of the muscles of the abdomen, trunk and extremities also produces marked weakness of the muscles of swallowing and often of respiration In addition, d-tubocurarine occasionally causes a fall in blood pressure and sometimes bronchoconstriction, probably due to the release of a histamine-like substance from the tissues Tetraethylammonium also has been somewhat limited in its efficacy, as a result of its short duration of action and some unexplained variations in its effects

In a search for new blocking agents Paton and Zaimis have studied the pharmacologic properties in animals of the bis-trimethylammonium series of compounds in which the only variation is the length of the polymethylene chain connecting the two quaternary ammonium groups They synthesized the compounds containing 2 through 12, and 18, carbon atoms in this chain, and referred to each compound by the number of carbon atoms in the chain Their animal work showed that the C 10 compound is a neuromuscular blocking agent which appears to have relatively less effect than d tubocurarine on the muscles of respiration, and less histaminic effect The C-5 compound was found to be a ganglionic blocking agent, similar in its effects to tetraethylammonium, but more potent and longer acting

The administration of the C-10 and C-5 compounds to human subjects has indicated that they will probably be clinically useful The intravenous administration of 5 mg of C-10 to normal human subjects resulted in weakness of the extraocular, facial, jaw, neck, and trunk muscles and almost complete paralysis of the muscles

of the extremities This weakness could be maintained by the intravenous injection of 0.5 mg of C-10 at five minute intervals When sufficient C-10 had been administered to cause almost complete paralysis of the muscles of the extremities there was relatively slight weakness of the muscles of swallowing, speech and respiration In contrast, when sufficient d-tubocurarine was administered to produce only moderate weakness of the arm muscles, and slight weakness of the legs, there was marked impairment of swallowing and speech and slightly greater impairment of respiration than had been encountered at a greater degree of peripheral relaxation produced by C-10 The effective dose of C-10 was only one-third that of d-tubocurarine on either a weight or molar basis, and its action was slightly less prolonged C-10 produced no fall in blood pressure or other manifestation suggesting the release of a histamine-like substance C-10, like d-tubocurarine, produced a progressive decline in the muscle action potentials initiated by supramaximal nerve stimuli, but the mechanism of action of the two compounds does not appear to be the same

The blocking action of C-5 on autonomic ganglia has been studied by observing the effect of this compound on the blood pressure The administration of 5 to 12 mg of C-5 intravenously usually resulted in a postural hypotension in normal subjects and some hypertensive patients, and a prompt fall in blood pressure in some hypertensive patients Patients with fixed hypertension had the least response to C-5 or tetraethylammonium The effective dose of C-5 was only one-tenth to one-twentieth that of tetraethylammonium, and its action was more prolonged

*Dr Harvey* Dr Grob's paper is now open for discussion *Dr Holaday*, would you like to make any comments?

*Dr Holaday* The studies that have been made with this compound so far have not been carried to the degree that I think we should make any broad statements about its future value, but it seems to give sufficient promise that the anesthesiologists may find it a worthwhile substitute, at least in certain cases, for curare preparations that are now being used, inasmuch as it does not seem to have the histamine-like releasing property that curare preparations seem to have Other than that, in the field of anesthesia at least, its action seems to be very similar to that of curare, with perhaps the exception that it spares the muscles of respiration and muscles innervated by cranial nerves to a somewhat greater extent than does d-tubocurarine This may detract somewhat from its value in anesthesia, inasmuch as curare is not infrequently used in troublesome laryngospasms that may arise in the course of pentothal or cyclopropane induction If the property of this drug that has been described in the preliminary studies—that it does favor the muscles of respiration somewhat more than does the curare, as compared to its paralyzing effect on the muscles of the extremities—it also might have slight disadvantages as compared with curare, inasmuch as the abdominal muscles are the muscles that give the surgeon primary concern as far as relaxation is concerned As these muscles are innervated by the intercostal nerves, those supplying muscles of respiration, one may

have to encroach somewhat more upon the respiratory mechanism in producing adequate relaxation for anesthesia than would be necessary in the case of the use of this drug C-10 in combatting the bone-breaking convulsions of shock therapy as employed in the treatment of certain psychoses

*Dr Harey* These drugs are of more than usual interest as far as basic physiological mechanisms are concerned in that the C-10 compound is not antagonized at all by neostigmine, but curiously enough, if the animal has received d-tubocurarine prior to administration of the C-10, the blocking action is decreased

One of our main interests in this whole problem is that pain pathways have synapses too, and, perhaps, if we understand more about these drugs that affect synapses in various regions—and here we have two that are very closely related as far as the active chemical group is concerned, each of which has specific action on different synapses—as we learn more about this action it may lead us to a drug which is a specific blocker of pain

*Dr Rich* May I ask one question? I understood Dr Grob to say that the injection of d tubocurarine produces wheals and acidity Can this be neutralized or prevented by benzamine? I would like to ask also whether increased gastric acidity accompanies the injection

*Dr Grob* We have not studied the effect of antihistaminic agents on the increased secretion of gastric acid induced by d-tubocurarine As you have indicated, the histamine induced secretion of gastric acid is said to be not inhibited by antihistaminic agents, so that a study of this kind might throw added light on the resemblance between the histamine-like substance liberated after d-tubocurarine itself There is no evidence that this substance is identical with histamine, but in the studies performed thus far it has had similar effects

*Dr Wilkinson* Is there any correlation between the response that hypertensive patients have with these drugs and the benefit they may expect to gain from sympathectomy?

*Dr Grob* We haven't given a sufficient number of patients C-5 to enable us to make such a comparative study Tetraethylammonium has been given to a large number of patients by other investigators prior to sympathectomy and there has been conflicting evidence as to its value in predicting the success of that operation Some investigators say that they cannot predict the value of sympathectomy by the response to tetraethylammonium Others, who appear to have been more careful in studying the effect of tetraethylammonium on the blood pressure in the erect, as well as the supine position, feel that there is frequently a correlation between the effects of tetraethylammonium and of sympathectomy on the blood pressure

*Dr Harey* Dr Thomas, would you like to make any comments?

*Dr Thomas* I would certainly like to point out that the results of the sympathectomy operation depend on the clinical condition of the patient

*Dr Harvey* The next two papers form a group and I think we will hear both of them before the discussion is opened. The first of these will be given by Dr John Seed and is on "The Mode of Action of 933-F Benzodioxine"

*On the Mechanism of Action of 933F* JOHN C SEED AND EVAN CALKINS

Piperidinomethyl-3-benzodioxane (933F) blocks temporarily the vasoconstrictor action of epinephrine but does not block the vasodilator action. Hence, after 933F epinephrine causes a fall in blood pressure, the fall being due primarily to vasodilation in striated muscle. Norepinephrine has no vasodilator action, so that its pressor action is blocked but not reversed by 933F. Most effects of epinephrine, such as those on the intestine, on the isolated vascular bed of nose and spleen, and on the blood sugar are blocked but not reversed. The accelerating effect of epinephrine on the denervated mammalian heart is not blocked. 933F blocks the action of sympathin but not the effects of sympathetic stimulation. 933F blocks the action of other pressor amines to a varying degree.

Compounds related to 933F which block the pressor action of epinephrine have in common a benzene ring linked to an oxygen which in turn is linked to an ethyl amine. These synthetic compounds have a common spatial configuration with the ergotoxine and yohimbine alkaloids. This structure consists of an oxygen linked to two carbons and separated by a distance equivalent to two carbon atoms (about 4.0–4.5 Angstroms) from a trivalent nitrogen. In the ergotoxine alkaloids the configuration exists in the amide side chain. Alterations in the ergotoxine molecule which affect this configuration abolish epinephrine-blocking activity whereas alterations in the lysergic acid part of the molecule do not. The change from yohimbine to yohimbic acid affects this configuration and also abolishes epinephrine-blocking activity.

It is postulated that compounds with this configuration compete with epinephrine for an "active surface." This "active surface" contains receptors for the alcoholic hydroxyl group and for the amine group in epinephrine. The competition between epinephrine and the blocking compounds is for this alcohol-amine receptor. It is proposed that epinephrine exerts its action by first combining with the "active surface" and then exerting an effect. If the "active surface" is occupied, then epinephrine cannot combine with it and exert an effect. It is postulated further that dibenzyl- $\beta$ -chlorethylamine (Dibenamine), which irreversibly blocks the pressor action of epinephrine, combines with or near this same alcohol-amine receptor. If the receptor is occupied already by another blocking compound, such as 933F, then Dibenamine should be unable to combine with the receptor. By testing whether the epinephrine blockade after an injection of 933F followed by Dibenamine is reversible or irreversible one can determine whether 933F or Dibenamine combined with the receptor.

5 mg/K of 933F completely prevents 10 mg/K of Dibenamine from blocking the pressor action of epinephrine in the dog anesthetized with sodium pentobarbital

*Dr Harvey* The final paper is on "Clinical Experience with 933-F in Hypertensive Patients" The paper will be given by Dr George Dana

*Clinical Experience with Benzodioxane* DR GEORGE W DANA AND DR EVAN CALKINS

Clinical experience with Benzodioxane, 933F, in 67 hypertensive patients is reported In each adult case, 20 mgms of the drug in saline, was given intravenously during a two minute period In no instance was there any lasting discomfort The usual coincident signs of the test were tachycardia, flush, and sighing respirations

Sixty-five cases gave what was interpreted as a negative Benzodioxane test, namely, a variable rise in blood pressure In the majority of these sixty five cases, the referring physician suspected that the cause for the hypertension might be a chromaffin tumor

One patient is described with sustained hypertension and symptoms of nausea, and bouts of perspiration, and weakness, whose blood pressure fell from 170/120 to 92/60 in three minutes following injection of 933F Operation revealed a pheochromocytoma of the left adrenal

In a second patient, aged six, suffering with sustained hypertension, the blood pressure fell from 200/145 to 160/100 in two minutes, following injection of Benzodioxane At operation a large cell neuroblastoma was encountered, incorporating the left adrenal, and enclosing the upper pole of the left kidney This test was interpreted as a false positive, and is yet to be adequately explained

The greatest potential value to the clinician of 933F appears to be in determining whether sustained hypertension is due to a pheochromocytoma

Further experience with this drug will undoubtedly define its diagnostic usefulness more accurately, but even now the Benzodioxane test can be included appropriately in the survey of hypertensive patients

*Dr Harvey* These two papers are now open for discussion Dr Howard, would you like to make any comments?

*Dr Howard* Dr Pincoffs, here, was the first to diagnose one of these cases Perhaps he would start the discussion

*Dr Pincoffs* We have had a limited experience with the benzodioxane test In a series of approximately 44 cases, we have not encountered a case of pheochromocytoma I feel, incidently, that the author of this interesting paper was fortunate to have discovered one of these rare tumors in his series since the incidence is



certainly not high enough to have made this probable. Of course the author's cases were selected from those who clinically suggested pheochromocytoma.

We experienced the same difficulty that has been mentioned,—that of interpreting the meaning of the rise in blood pressure following the introduction intravenously of benzodioxane. We assumed that perhaps this rise in pressure was of psychic origin. It was found that when the procedure of the test was altered by establishing first a slow intravenous infusion of normal saline with the apparatus out of sight behind the patient and then introducing the benzodioxane by injection into the rubber tubing of the infusion set without the patient's knowledge, the rise in blood pressure was minimized. We did not infer however that in some instances these pressure rises may not be due to drug action. We did feel that in some there was a distinct psychic component.

*Dr Harvey* With such a pharmacological evening as this, I am sure Dr Blanchard is anxious to add to this discussion.

*Dr Blanchard* Just as a matter of historical interest I might point out that the technic used by Dr Seed, namely, calculation of the distance between two or more atoms of a drug, is an aspect of the theory of drug activity employed by chemists for a number of years past. Fourneau actually employed this concept in developing drugs of the 993-F series, only what he was trying to design was an antimalarial drug. These drugs turned out to exhibit the activity that we have heard about this evening, after remaining on the shelves for some time.

*Dr Harvey* Are there any further questions or comments? The meeting is closed.

# ON THE RELATION OF POTASSIUM TO THE NEUROLOGICAL MANIFESTATIONS OF HYPOCALCEMIC TETANY<sup>\*</sup>

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There are many factors recognized to be involved in the production of the neurological manifestations of tetany among which hypocalcemia is generally considered to be the most important. However, under certain circumstances tetany may appear with only slight decreases in the total serum calcium. In other situations more considerable depressions of serum calcium may be present without tetany. Since neuromuscular irritability is influenced by the concentration of ionized calcium, some of these discrepancies are readily explained by the presence of factors which alter the proportions of ionized to unionized or bound calcium in the serum. The serum protein level is of the first importance in this respect. The hydrogen ion concentration of the blood is also significant both in altering the degree of ionization of calcium in the serum and in influencing neuromuscular irritability directly. Alkalosis decreases the proportion of ionized calcium and at the same time directly increases neuromuscular irritability. The development of tetany after vomiting and hyperventilation is due largely to alkalosis. In addition to the absolute level, the rate of change in serum ionized calcium influences the production of symptoms. A sudden reduction in serum calcium from a high level, as after parathyroidectomy for hyperparathyroidism, is more likely to result in tetany than is the same degree of reduction occurring over a considerable period of time.

There are other factors involved in the production of tetany which may not be mediated through changes in the serum calcium. The conversion of latent to overt local or generalized tetany as in the

<sup>\*</sup> A preliminary report was made to the Southern Society for Clinical Research, New Orleans, La., January 27, 1948 and published in Abstract Form, American Journal of Medicine, 4, 455 (1948).

Trousseau phenomenon is an example. Here the effects of ischemia apparently summate with the low calcium peripherally to convert latent to frank tetany. Epinephrine injected intravenously or intra-arterially similarly will incite tetanic phenomena either generally or locally in the presence of a depressed serum calcium (1). We have seen carpopedal spasm induced in a patient with latent tetany following the subcutaneous injection of histamine and of urocholine. Finally emotional factors possibly acting through sympathetic discharges, hyperventilation or hysteria may precipitate or simulate tetany. In none of these situations has a further change in serum calcium been demonstrated to be responsible for the manifestations.

Experimentally and in the absence of changes in the serum calcium, phenomena which may be interpreted as tetany have been produced by magnesium depletion with resulting low serum magnesium (2). One case of pure hypomagnesium tetany has been reported in the pediatric literature (3). In the presence of a low concentration of calcium in the serum, a concomitant decrease in serum magnesium might be expected to precipitate tetany. As yet there have been no studies to demonstrate this point.

It is clear, therefore, that there are many other factors in addition to the level of the serum calcium which are concerned with the actual precipitation of the neurological phenomena of tetany. The purpose of this report is to describe one more factor which enters into the problem of tetany and which has not received attention in the clinical literature, namely the level of the serum potassium.

The physiological antagonism of calcium and potassium ions in neuromuscular action has been long recognized. Within certain limits an increase in nerve irritability *in vitro* can be produced by decreasing the calcium ion concentration or increasing the potassium ion concentration. *In vivo*, diminution of the serum potassium is associated with muscular weakness or paralysis. Depression of serum calcium is associated with tetany. Elevations in calcium may result in some muscular weakness, while elevation in potassium may be followed by either an increase in irritability or weakness.

An opportunity to study the interrelations of calcium and potassium in tetany was afforded us by the observation of two patients with hypocalcemia who developed the low potassium syndrome. In one

tetany could be induced or abolished almost at will by altering the level of serum potassium without changing serum calcium

The first of the two patients was a 28 year old, single, dwarfed, white female with a long history of diarrhea of undetermined etiology, but possibly pancreatic in origin. She had roentgenological and blood chemical findings consistent with a diagnosis of osteomalacia, i.e. normal serum calcium, low serum phosphate and elevated alkaline phosphatase. Four months before she was seen by us the patient was subjected to a parathyroid exploration with removal of parathyroid tissue because some observers suspected hyperparathyroidism. Subsequently the serum calcium fell from 9.5 to 7.6 mg per 100 ml and her serum phosphate rose from 1.6 to 2.2 mg per 100 ml. About this time the first evidence of diabetes mellitus developed but the disease was not recognized until  $3\frac{1}{2}$  months later when the patient returned in coma with severe acidosis. Her blood sugar was 398 mg per 100 ml and  $\text{CO}_2$  combining power 14 vol per cent. She was treated vigorously with insulin, intravenous saline, and glucose in the conventional manner but was brought out of ketosis with difficulty. Twenty-four hours after admission when her diabetes had been fairly well controlled she complained of severe weakness and dyspnea with inability to lift her head or arms from the bed. Deep tendon reflexes were depressed. The low potassium syndrome following treatment of diabetic acidosis and accentuated by chronic diarrhea was suspected and confirmed by the finding of a serum potassium level of 1.4 milli-equivalents per liter. Serum calcium at this time was 7.2 mg per 100 ml. No evidence of tetany was noted. She was given 3 grams of potassium chloride intravenously as a 1 per cent solution following which the paralysis promptly disappeared but she began to complain of severe cramps in the legs. She was noted to have a positive Chvostek sign. The significance of this finding was not appreciated at this time so that correlative studies of serum calcium and potassium levels were not made, nor were careful notes kept of changes in signs and symptoms referable to tetany. She was treated with oral calcium (7.2 grams calcium lactate and 12 grams dicalcium phosphate daily), 1.8 grams of potassium chloride a day and vitamin D (10,000 units per day). Six days after these symptoms were first noted the serum potassium was 2.1 milli-equivalents per liter and serum calcium 6.0 mg per 100 ml. The patient complained of pain

in the legs but had neither unusual weakness nor signs of tetany. The next blood chemical determinations recorded were 6 days later when the serum potassium was 4.0 milli-equivalents per liter, and the serum calcium 6.5 mg per 100 ml. Severe cramping pains in the legs were present and the Chvostek sign was positive. Shortly after this all these symptoms subsided, her serum calcium and potassium having risen to normal levels. The real significance of these findings, however, was not appreciated until 3 months later when the same phenomena were noted in a second patient and an opportunity was afforded to study the calcium-potassium antagonism in greater detail with regard to neuromuscular irritability and changes in the electrocardiogram.

This patient was a 32 year old white married female who had had three previous admissions to Duke Hospital with the diagnosis of non-tropical sprue. During previous admissions and on visits to the clinic the patient had been noted frequently to have symptoms of tetany requiring intravenous injections of calcium for relief. She was first seen by us on her fourth admission, which was occasioned by a relapse in her disease. The patient was greatly emaciated on admission, weighing only 76 lbs. She complained of weakness and on the morning after admission it was noticed that she was unable to lift her arms to feed herself. Hypokalemia was suspected because of these symptoms in a patient with protracted diarrhea and was confirmed by finding a serum potassium of 1.92 milli-equivalents per liter. The serum calcium was 5.7 mg per 100 ml, total protein 4.6 grams per 100 ml with 2.1 grams of albumin and 2.5 grams of globulin. There were no signs of tetany at this time. She was given 300 ml of 1 per cent potassium chloride intravenously. By the time the infusion had ended she had developed severe carpopedal spasm, which slowly subsided during the next 24 hours. The serum calcium was now 5.7 mg per 100 ml, the serum potassium 2.18 milliequivalents per liter and there were signs of latent tetany, i.e. fasciculation and a positive Trousseau's sign. During the ensuing three months repeated opportunities were offered to observe this phenomenon and a series of studies were carried out as described below.

#### METHODS

All chemical determinations were carried out by the clinical laboratory of the Department of Biological Chemistry under the direction of

Dr H M Taylor Serum potassium and sodium levels were determined with a Barnes Flame Photometer We are indebted to the American Cyanamid Company for this instrument (4) Ionized calcium was estimated from the total calcium and the total protein levels by the use of the nomogram of MacLean and Hastings (5)

Electrocardiograms were recorded with a Sanborn portable cardiette Q-T intervals were measured and compared to the maximum normal for the patient's sex, age and cycle length, as determined from the table of Ashman and Hull (6)

### RESULTS

Figure 1 records the fluctuations in the patient's symptoms of neuromuscular irritability as correlated with changes in serum calcium and potassium Concomitant levels of other significant blood constituents are also noted The solid blocks represent rough estimates of manifestations of either muscular weakness or increased irritability graded from 1+ to 4+ The quantitation of weakness was difficult to estimate and arbitrary Tetany was scored as 1+ when latent, 2+ fasciculations, 3+ mild carpopedal spasm, and 4+ severe carpopedal spasm On examining the chart, it will be noted that, by and large, fluctuations in neuromuscular symptoms occurred in relation to the serum potassium levels rather than to the ionized calcium The latter actually changed very little during these observations Changes in total calcium paralleled those in total protein, both reaching their highest levels following a period of therapy with concentrated serum albumin between the 23rd and 33rd days It is realized, of course, that the estimation of ionized calcium by the nomogram of MacLean and Hastings is only a rough approximation under these circumstances, particularly when simultaneous changes in acid-base balance occur The serum calcium and potassium levels were lowest on admission each time, but due to intravenous potassium chloride administration and the alleviation of the severe diarrhea by therapy, the potassium levels rose more quickly than the calcium This caused the appearance of the most severe symptoms of tetany during the first few days of each admission Although the calcium levels rose somewhat with intensive therapy with vitamin D, large amounts of calcium lactate by mouth and frequent intravenous injections of calcium gluconate, they never reached normal levels except immediately after the intravenous medica-

tion The serum potassium levels were usually moderately to severely depressed, except during or immediately after intravenous potassium injections During the second admission when the serum calcium levels had become stabilized at a higher level tetany became minimal The serum potassium, however, had fallen from its highest level Subsequently both the calcium and potassium rose slightly, but despite this, symptoms of tetany became more severe It will be noted that at the time the level of ionized calcium had become somewhat more

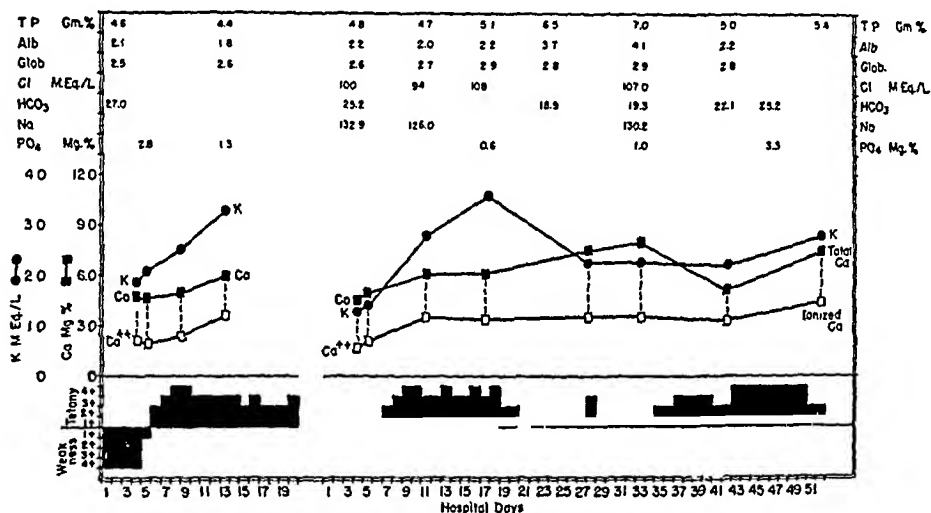


FIG 1 The relation of the serum potassium and calcium levels to neuromuscular irritability in a case of sprue See text for description

stable, the patient was in mild acidosis with a  $\text{CO}_2$  combining power value of 18.9 millimols per liter This would tend to increase ionized calcium, introducing an error into the estimation from the nomogram Tetany increased in the face of an apparent rise in ionized calcium when the  $\text{CO}_2$  combining power had returned to normal, i.e. 25.2 millimols per liter

Figure 2 records the same serum calcium and potassium data as in the previous figure, correlating them with the changes in the three standard leads of the electrocardiogram The black bars in the middle part of the figure indicate the maximal duration of Q-T interval for the age, sex and pulse rate of the patient, while the total bar represents the Q-T interval found on each examination Note that at the beginning

of each admission when the serum potassium and calcium levels were lowest, the electrocardiograms showed the characteristic changes attributed to hypokalemia. These include depression of S-T segments, low T waves, and prolongation of the Q-T interval (7). With the increase in serum potassium levels, these abnormalities disappeared. Subsequent electrocardiograms, not shown on the chart, have been entirely normal when the serum potassium levels have been 3.5 to 4.0 milli-equivalents per liter.

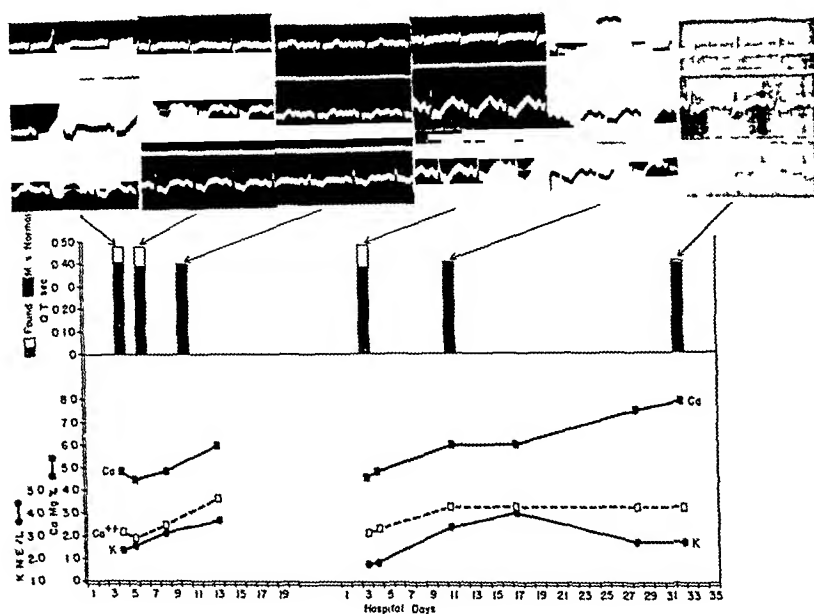


FIG 2 Electrocardiographic changes and serum potassium and calcium levels in a case of sprue. See text for description.

Figure 3 illustrates the effects of intravenous potassium chloride administration on the signs and symptoms of tetany, the serum calcium and potassium, and the electrocardiograms. Potassium chloride was injected as an 0.5 per cent solution in normal saline over the course of two hours. At the beginning of the infusion the patient exhibited a severe flacid paresis, being unable to lift her head or her arms from the bed. Her serum potassium level was 1.5 milli-equivalents per liter at this time and her serum calcium 4.5 mg per 100 ml. The electrocardiograms showed evidence of hypokalemia, i.e. a prolonged Q-T interval, depressed S-T segments and inverted or flat T waves. Within 30



minutes of the beginning of the infusion the paresis had subsided being replaced by increasing signs of tetany. The latter became very severe a little over an hour after the infusion was begun and lasted until the conclusion of the infusion, following which they subsided to some degree. Note that the serum calcium remained fixed while the serum

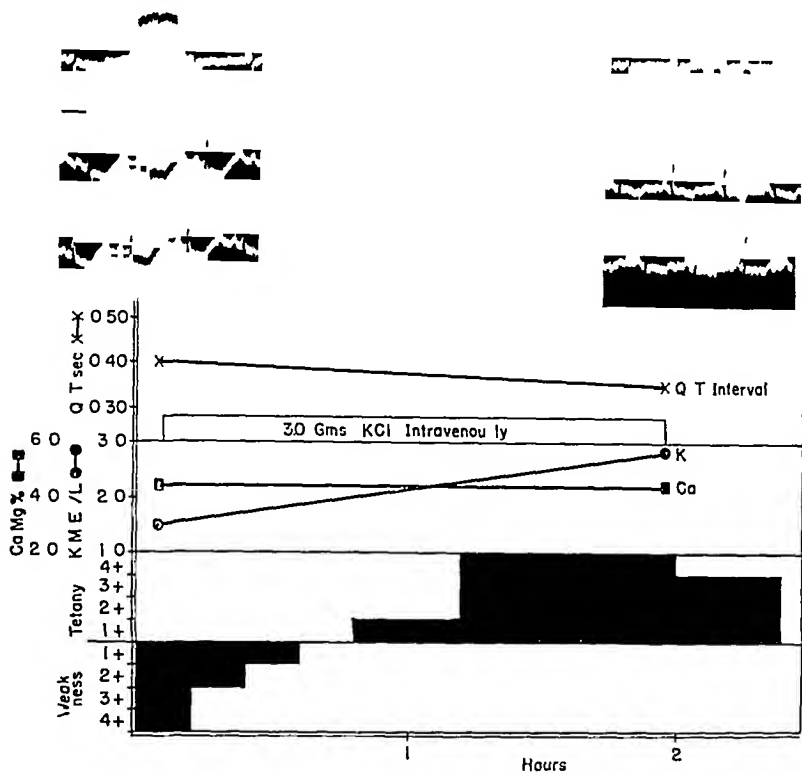


FIG 3 Effects of intravenous administration of potassium chloride on neuromuscular irritability, serum potassium and calcium levels and the electrocardiogram when patient's symptoms were predominantly those of hypokalemia. Note development of tetany with no change in serum calcium.

potassium increased from 1.5 to 2.9 milli-equivalents per liter. The Q-T interval of the electrocardiogram returned toward normal. This experiment was repeated 3 times with identical results except that on one occasion the calcium level decreased from 5.3 to 4.8 mg per 100 ml during the infusion (Fig 4). The serum potassium level usually had returned to or near the initial level when next determined, 12 to 24 hours later. On one test the intravenous infusion of 3 grams of potas-

sium chloride in 1500 ml of saline over 5 hours did not abolish the symptoms of hypokalemia or elevate the serum potassium. Prior to the infusion the serum potassium was 1.28 milli-equivalents per liter and at the end 1.54 milli-equivalents per liter, an insignificant change. There was no change in serum calcium. On another occasion when there was latent tetany (day 33 of 2nd admission—Fig 1), slow infusion of 3 grams of potassium chloride in 1300 ml of saline also had no effect

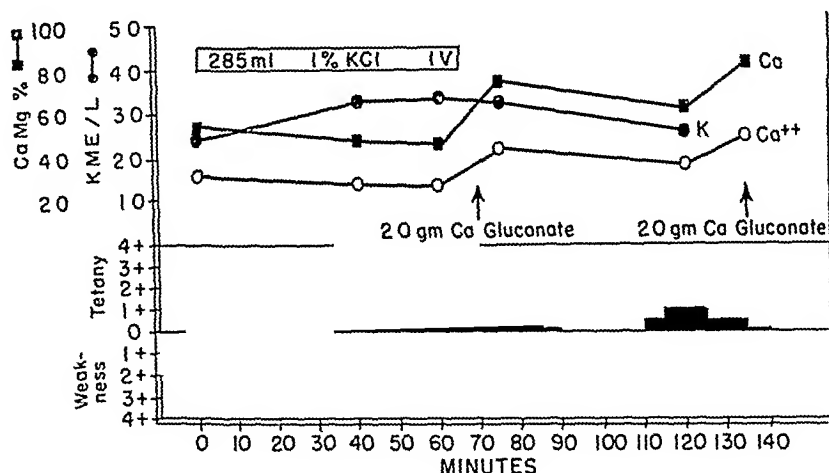


FIG 4 Production of tetany by intravenous administration of potassium when patient was suffering from hypokalemia with muscle paresis. Abolition of tetany by calcium gluconate injection intravenously. Note that tetany developed with no appreciable change in calcium and that tetany began to subside after calcium administration before a significant decrease in the serum potassium occurred.

either on symptoms or electrolyte levels. Symptoms and signs of tetany occurred after intravenous potassium infusion only when there was a rise in serum potassium and either no change or a fall in serum calcium.

Figure 4 illustrates the production of tetany by infusion of 285 ml of 1 per cent potassium chloride in one hour and its alleviation by calcium gluconate before the serum potassium level had changed appreciably. In this experiment the serum calcium level decreased slightly so that it is not possible to attribute the tetany solely to changes in serum potassium.

Figure 5 depicts the effects of 6.0 grams calcium gluconate adminis-

tered intravenously on the signs and symptoms of tetany, the electrocardiogram, and the serum electrolyte concentrations at a time when the patient had severe spontaneous tetany. Tetany was abolished with the rise in serum calcium but there was no change in the Q-T interval of the electrocardiogram or in the serum potassium level.

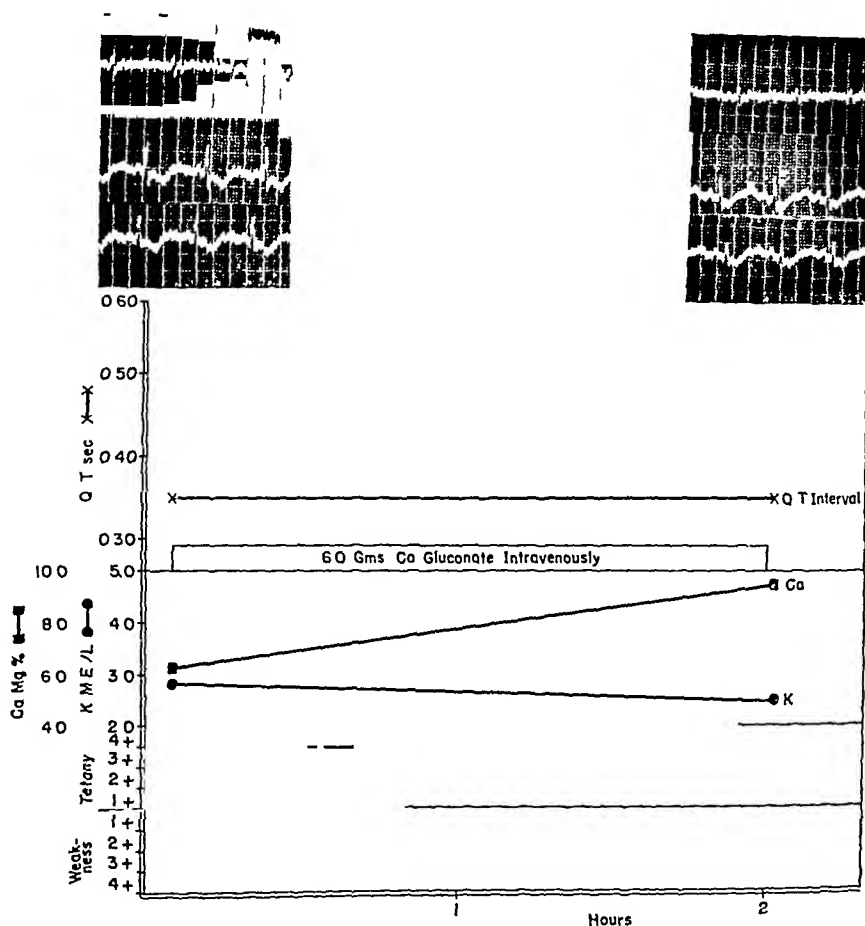


FIG 5 Effects of intravenous calcium gluconate infusion on the signs of tetany, serum calcium of potassium levels at a time when tetany predominated

On three occasions 3 grams of potassium chloride and 6-8 grams of calcium gluconate were administered simultaneously by slow drip. In all cases the calcium effect predominated, signs and symptoms of tetany decreasing but never completely disappearing, in contrast to the effect of calcium alone. Calcium levels rose considerably each time.

Serum potassium was unchanged in two experiments while in the third the determinations unfortunately were unsatisfactory

Two unsuccessful attempts were made to convert local latent to overt tetany by injection of potassium chloride into the brachial artery at a time when the patient exhibited a positive Trousseau's sign. 20 ml of a 0.01 per cent potassium chloride solution in 0.8 per cent NaCl were injected slowly. Marked flushing of the hand occurred. On the first trial the serum calcium was 7.9 mg per 100 ml with a total protein of 7.0 grams per 100 ml and an arterial potassium level of 2.32 milli-equivalents per liter before injection and 2.56 milli-equivalents per liter in the brachial vein on the same side after injection. On second trial the serum calcium was 7.2 mg per 100 ml with a total protein of 5.4 grams per 100 ml, while the arterial potassium was 2.82 milli-equivalents per liter before and 3.20 milli-equivalents per liter afterwards in the venous serum. Since in both experiments the serum calcium levels were somewhat higher than usual we do not consider these results decisive. Subsequently the patient has had a remission in her disease and her electrolyte values have returned to normal and no further opportunity has been presented us to study these phenomena.

#### DISCUSSION

Alternations in a serum potassium as a factor in tetany have received little attention in the clinical literature. To our knowledge, the reports of Harrison, Harrison Tompsett, and Barr (8, 9) on serum potassium in sprue contain the only mention of this relationship. These workers commented in passing that when their patients were suffering from hypokalemia they showed none of the manifestations of tetany even though their serum calcium levels were low. They made no observations on the effects of potassium administration. Since our studies were completed two interesting reports have appeared in the veterinary literature describing the induction of tetany in hypocalcemic cattle by the administration of potassium (10, 11). In the syndrome of "grass staggers" due to grazing wheat, Harbaugh and Dennis noted that the serum calcium ranged from 3.3 to 6.6 mg per 100 ml in 5 cattle and the serum potassium from 9.6 to 26.6 mg per 100 ml, most of the potassium values being below normal. The animal with the highest potassium level showed spontaneous tetany and had a potassium-cal-

cium ratio of 4.03 (Normal 1.53) Tetany was induced in the asymptomatic animals by administration of potassium chloride by stomach tube until the K/Ca ratio was about 3.35 When the calcium was reduced by giving oxalates, tetany developed with a ratio as low as 2.62 and a serum potassium level of 9.5 mg per 100 ml These findings in cattle are very similar to those in our patient with sprue except that we could not establish a clear relationship between the serum potassium-calcium ratio and occurrence of tetany

Beyond the potassium ion concentration being a factor in nerve-muscle irritability, our observations do not clarify the exact role of potassium in the body economy with respect to tetany These data do not permit us to draw any conclusions as to the relative importance of the extra or intracellular potassium ion concentrations in the phenomena described The fact that tetany was observed after the administration of potassium only when there was a rise in serum potassium does not prove the latter to be the significant change The serum potassium may begin to rise only after the depleted cellular depots have been replenished to a sufficient degree The failure to alter the serum potassium significantly on several occasions even after the administration of 3 grams of potassium chloride would support this interpretation It should be noted that the levels of serum potassium were always low in this study and that the concentrations of potassium associated with tetany were still well below normal We have no evidence that elevation of serum potassium above normal will induce tetany if serum calcium levels are not reduced However, there is a suggestion of this in the experiments of Harbaugh and Dennis on cattle (10, 11) These workers reported neuromuscular reactions they interpreted as tetany after administration of potassium chloride without a reduction in serum calcium While muscle twitching has been noted in some patients with elevated potassium levels, to our knowledge typical carpopedal spasm, Trousseau's phenomenon and Chvostek's sign have not been described in these patients in the absence of either low calcium or alkalosis These observations suggest that from the clinical standpoint the potassium changes are of greater significance in a negative sense, i.e., a low potassium inhibits hypocalcemic tetany rather than that a high potassium is conducive to tetany when the serum calcium is normal or even slightly depressed However, conclu-

sive data on this point are not available. The possibility that the administration of potassium induced tetany in our patients by any means other than changes in concentrations of potassium seems unlikely but cannot be ruled out with certainty. In one of our experiments intravenous potassium injection resulted in a decrease in serum calcium level from 5.3 to 4.8 mg per 100 ml, a change of questionable significance but a phenomenon which has been reported before (12). However, it seems unlikely that such severe tetany as was observed would be induced by such a small change in calcium. Furthermore, the change in calcium was noted only once in six experiments. Finally, the spontaneous increase in serum potassium that occurred in the hospital was associated with increasing symptoms of tetany while the calcium either remained constant or increased slightly. No measurements of blood pH or  $\text{CO}_2$  combining power were made before and after potassium administration, but there is no evidence to suggest that significant shifts would have occurred. Darrow has reported on the association between alkalosis and hypokalemia (13).

One may speculate as to whether the changes in serum potassium bear any relation to previously described observations on tetany. The Trousseau phenomenon by which latent hypocalcemic tetany is converted to overt tetany by the application of a pressure cuff to the arm is a case in point. Under conditions of hypoxia potassium is released from cells. A local increase in potassium at the neuromuscular junctions after the application of the cuff might be suggested as responsible for the local spasm. Our attempts to demonstrate such a phenomenon by the intra-arterial injection of potassium were unsatisfactory. The studies of Lewis (14) and of Kugelberg (15) suggest that the Trousseau phenomenon is due to changes in the proximal part of the nerve rather than at the neuromuscular junctions and may be produced by ischemia of the nerve trunk itself without concomitant ischemia of the rest of the arm. The possibility of local changes in potassium and calcium occurring in the involved stretch of nerve, however, cannot be ruled out by any available data. A similar question may be raised with respect to the induction of local tetany by intra-arterial injection of epinephrine (1), again, without an answer. Epinephrine will cause a release of potassium from the muscle locally (12), although its injection intravenously is associated with a decrease in serum potassium levels, possibly

due to changes in the liver. Until it is possible to measure the electrolyte concentration at the site in the neuromuscular system where the tetanic phenomena are initiated one can only speculate that changes in the concentration of potassium as well as calcium or changes in their proportions are essential features of tetany and that the production of tetany by the various known clinical maneuvers may be mediated through such changes.

These observations on the suppression of hypocalcemic tetany by hypokalemia undoubtedly have bearing on the variability of appearance of tetany in the presence of low serum calcium associated with different clinical conditions. With the increasing frequency of measurement of serum potassium in clinical practice one may anticipate more correlations of this type. In sprue and steatorrhea tetany is a very variable phenomenon, while hypocalcemia is a very frequent feature of the more severe cases. In the infantile diarrheas, particularly in the postacidotic stage (16) both hypocalcemia and hypokalemia occur. Rapaport comments on the relative infrequency of the peripheral manifestations of tetany in infantile diarrhea despite low calcium values. He attributes this to a generally lower incidence of peripheral tetany as contrasted with central excitability in the presence of hypocalcemia in infants compared to older children. Since most of the data on infantile tetany have been derived from cases of diarrhea, one wonders whether concomitant hypokalemia may not be a masking factor. It is not possible to make a definite decision about this from Rapaport's data since not enough simultaneous potassium and calcium determinations were reported. Chronic nephritis is another condition associated with hypocalcemia in which changes in serum potassium occur. Since both hyper- and hypokalemia occur in this condition (7, 17) one may anticipate that both inhibition and excitation of tetany with the same calcium levels may be seen. We have not had the opportunity to study any such cases.

In view of the severe signs and symptoms of tetany noted in one of our patients following potassium administration, a word of caution is necessary concerning too enthusiastic treatment of the low potassium syndrome under circumstances where there may also be hypocalcemia. When both are present simultaneous administration of calcium and potassium is recommended. In our experience with steatorrhea it is much easier to correct the hypokalemia than the hypocalcemia.

The abnormalities in the electrocardiograms noted in this study were largely corrected by the administration of potassium, suggesting that their origin was determined by potassium deficiency. The changes were of a type repeatedly described in the literature (7) as associated with hypokalemia. Certain changes, however, such as a prolonged Q-T interval, have been described under a variety of circumstances other than hypokalemia, some of which were common to our patient with sprue. Thus a prolonged Q-T interval has been noted with hypocalcemia (18, 19, 20), with acidosis (21, 22), and with severe undernutrition (23, 24), all of which were present to varying degrees in our patient. The prompt shortening of the Q-T interval following the intravenous administration of potassium suggests potassium deficiency as being responsible for this abnormality. There are no data available to demonstrate whether potassium will shorten the prolonged Q-T segment in other conditions in which the serum potassium level is normal. Hypocalcemia did not seem to be a significant factor since the Q-T changes returned to normal both in the acute experiments and during prolonged observations at times when no significant increases in serum calcium had occurred. Furthermore, it has been pointed out recently (25) that the prolongation of the Q-T interval in hypokalemia is due primarily to a widening of the T wave, whereas in hypocalcemia the Q-T prolongation occurs because of an increase in the RS-T segment in the presence of a normal T wave. In our case, widening of the T-wave accounted for the prolonged Q-T interval and the return to normal was associated with changes in the T wave along. This may be taken as further evidence that hypokalemia was the chief determining factor in the electrocardiographic changes described. The acidosis in our patient was very mild and was present at a time when the Q-T interval was normal. The undernutrition was a relatively constant factor during most of these studies.

#### SUMMARY

- 1 Two cases are reported in which symptoms and a sign of hypocalcemic tetany were masked by the simultaneous presence of hypokalemia.
- 2 With the serum ionized calcium remaining relatively constant, fluctuations in signs and symptoms of tetany were correlated with spontaneous increases or decreases in serum potassium.



3 In the presence of hypokalemia and hypocalcemia without tetany, severe tetany could be induced at will by elevation of the serum potassium by intravenous infusion of potassium chloride. Tetany so induced could be abolished by administration of calcium salts without changes in serum potassium or disappeared spontaneously when the serum potassium returned to its initial low level after infusion without changes in serum calcium.

4 The electrocardiographic abnormalities noted were characteristic of hypokalemia and were abolished by the administration of potassium but not by calcium.

5 The significance of the serum potassium level in the relation to neurological phenomena of tetany are discussed in light of these data.

6 The danger of too vigorous therapy with potassium in the presence of hypocalcemia is emphasized.

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# AN INVESTIGATION INTO THE PRESENCE OF ANTIBODIES AND HYPERSENSITIVITY IN THE ENCEPHALITIS PRODUCED EXPERIMENTALLY BY THE INJECTION OF HOMOLOGOUS BRAIN SUSPENSIONS

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The objects of the experiments to be described were

- 1 To produce an encephalitis in guinea pigs and rabbits by the injection of homologous brain plus adjuvant This had already been done by Freund et al (1) and Morrison (2) respectively, on the above animals
- 2 To attempt to correlate the presence of circulating antibodies with the clinical signs and histological findings of encephalitis
- 3 To attempt to passage circulating or tissue antibody

In 1932 Hurst (3) produced paralysis in rabbits following repeated subcutaneous and intramuscular injections of heterologous brain, but he found no microscopic lesions in any of his paralyzed animals

Rivers et al (4) in 1933 produced paralysis and perivascular demyelination in two out of eight monkeys, and Rivers and Schwentker (5) in 1935 produced signs of involvement of the CNS in six, and found similar histological lesions in seven out of eight monkeys, both groups of workers using repeated intramuscular injections of heterologous brain

Later Ferraro and Jervis 1940 (6) by the prolonged subcutaneous injections of heterologous brain were able to produce paralysis in monkeys associated with perivascular demyelination

The above investigators used heterologous brain tissue in their experiments In 1947, using homologous tissues and Freund's adjuvant technique (7) (8), Kabat et al (9) and Morgan (10) demonstrated that paralysis and perivascular demyelination could be produced by the injection of brain tissue into monkeys, Morrison (2) produced paralysis and perivascular demyelination in rabbits using cord tissue, and

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Freund et al (1) produced paralysis and vascular lesions, but no demyelination, in guinea pigs using brain tissue

The production of complement-fixing antibrain antibodies in rabbits following the injection of heterologous brain has been demonstrated by Brandt et al (11) in 1926 and also by Witesbsky and Steinfeldt (12) in 1928, in 1933 Lewis (13), and in 1934 Schwentker and Rivers (15), also showed the presence of circulating complement-fixing antibrain antibodies in the rabbit after the injection of homologous brain tissue Kopeloff and Kopeloff (14) in 1944, using homologous brain tissue, produced complement-fixing antibrain antibodies in the monkey

To the author's knowledge there has been, however, little attempt to correlate the presence of circulating antibody with the appearance of paralysis or histological lesions following the injection of brain tissue by the various methods To date, the only one that is known is that of Freund et al (1) who mention that they found complement-fixing antibrain antibodies in guinea pigs treated with heterologous brain but they concluded that these antibodies were unrelated to the development of the encephalitis It is true that the complement-fixing and precipitin antibrain antibodies produced in rabbits by Schwentker and Rivers (15) by the intraperitoneal injection of homologous brain [(a) autolysed, (b) virus treated, and (c) alcoholic extracts with pig sera as a conveyor] were associated with paralysis and various non-demyelinated inflammatory lesions in the CNS, but these authors believed that the histological lesions and the paralysis were not directly related to the antigenicity of the brain emulsions injected Their reasons for this were that the histological lesions were not present in all the paralyzed animals and that they occurred in some of the non-paralyzed animals Further, they referred to the work of Hurst (3) who found that paralytic accidents may follow injection of substances other than brain, and they concluded that in their experiments the autolysed and virus treated brain injected may have contained toxic substances

Kabat et al (9) failed to passage the encephalitis experimentally produced in monkeys by the intravenous injection of two normal monkeys with sera, 35 and 40 ccs respectively, from affected animals, and similarly Morgan (10) failed to passively transfer the encephalitis agent from affected monkeys by injecting two monkeys each with 20 ccs of serum, intraperitoneally, and Freund's et al (1) attempts at passage

using sera from affected guinea pigs injected into guinea pigs also failed (details not given) However, none of the above investigators tested the serum used in the passage experiments to determine whether or not it contained demonstrable antibody

#### METHODS

Experiments I and III were concerned with guinea pigs, and II, IV and V with albino rabbits Approximate average weights of guinea pigs and rabbits was 370 and 2100 grammes, respectively

##### (a) *Preparation of guinea-pig antigen mixture*

*Experiment I* Under aseptic precautions the cerebrums (5 gms) of two guinea pigs, killed by exsanguination, were freed from meninges as far as possible, and were homogenized in a Waring Blendor with 50 ccs of normal saline containing 1% phenol to make a 10% emulsion Twenty ccs of this emulsion were slowly added to 20 ccs of warmed Falba,<sup>1</sup> 1 cc of each at a time, and mixed to a paste-like consistency in a mortar To this mixture 20 ccs of heat killed dried tubercle bacilli in suspension in light liquid paraffin oil (0.1 gm bacilli in 80 ccs oil) were added, and the 60 ccs of final mixture ground in a mortar until the whole was of a thin creamy consistency

The 60 ccs of brain antigen and adjuvant was heated to 56° for 45 minutes to prevent autolysis, and was stored in the deep freeze

*Experiment III* The preparation of antigen was essentially the same, but the saline emulsion was prepared without the addition of phenol and the final mixture was used immediately and not heated to 56° This was to eliminate any possibility of the denaturing effect of heat on protein or the production of any "unspecific" reaction by phenol, (11) (17)

##### (b) *Preparation of rabbit brain antigen mixture*

*Experiment II* The cerebrum (5 gms) of a rabbit which had been exsanguinated, was treated as described above in Experiment 1 for the preparation of guinea pig antigen, so as to make 60 ccs of antigen and adjuvant mixture

<sup>1</sup> Falba (Pfalz and Bauer Inc, New York) A hydrous wool fat-like absorption base

*Experiment IV* Sixty ccs of rabbit brain antigen and adjuvant mixture was made as described above in Experiment III for the preparation of guinea pig antigen

*Experiment V* The antigenic mixture was prepared in accordance with the directions given by Morrison (2) Two ccs of freshly ground spinal cord were emulsified with 4 ccs of a suspension of formalin killed tubercle bacilli in light liquid paraffin oil

#### *Preparation of Control Injection Mixture*

An adjuvant control injection mixture was prepared for each experiment by methods as described above under experiments I, II, III, IV and V except that in each case normal saline was substituted for the saline brain suspension of the test injection

#### *Technique of Injection*

In Experiments I, II, III and IV all animals were given at one time three deep subcutaneous nuchal injections of 1 cc of test mixture or control Each test animal, therefore, was injected with brain 0.1 gm, saline 1 cc, Falba 1 cc, paraffin oil 1 cc and tbc 0.0013gms Because of factors outside the author's control, the test (antigen) and control mixtures of Experiments I and II were left in the deep freeze 14 days before use

In Experiment V the rabbits received an injection of 0.1 cc of the test or control mixture into the pad of each foot, i.e. a total of 0.4 ccs at one time

#### *Skin Tests*

0.1 cc of 10% homologous brain emulsion was injected intradermally In Experiments I and II the animals were also tested by injection with 0.1 cc adjuvant control mixture The sites of injection were observed for the prompt anaphylactic type of reaction, and were examined 24 and 48 hours later

#### *Autopsy and Histology*

Animals were killed when they showed signs of paralysis or were moribund, or after a period of time considered to be sufficient within the limits of the experiment They were killed by exsanguination

through cardiac puncture and the blood kept for serology in the deep freeze. At autopsy the brain, cord and viscera and sciatic nerves were taken for histology and preserved in 4% formalin-saline. Paraffin sections were stained with haematoxylin and eosin. In addition frozen sections stained with Sudan III were made on all brains, and where indicated, Weils or Mahon's Myelin stain, Alzheimer-Mann, Holzer, Toluidine blue, Mallory's connective tissue, Van Geison and Dublin's reticulin and axis cylinder stains were carried out.

*Serology* (In all cases blood for serology was taken at time of death)

*Complement Fixation Tests* These were carried out on serum, which had been preserved in the deep freeze, using the Eagle modification of the Wassermann test as performed in the Wassermann Laboratory of the Johns Hopkins Hospital (20, 21). The antigen used was a 1-3% saline suspension of homologous brain and the tests were carried out at first qualitatively and then quantitatively.

*Flocculation Test* These were carried out using the Eagle Flocculation Test (22). The antigen was prepared by filtering a 10% saline suspension of homologous brain through filter paper and then centrifuging the filtrate at 2000 rpm for  $\frac{1}{2}$  hour, the supernatant fluid being used.

*Absorption Test* For both complement fixation and flocculation tests homologous brain or kidney suspensions were used, both being prepared in a manner similar to that used in preparing the original brain antigen suspension before addition of the adjuvant, except that the kidney was well perfused with saline before removal. For absorption  $\frac{1}{2}$  cc of test serum was mixed with  $\frac{1}{2}$  cc of 1/30 brain or kidney emulsion, placed in a water bath at 56° for 2 hours and then left overnight on the bench at room temperature. The following morning the tubes were centrifuged at 2000 rpm for  $\frac{1}{2}$  hour and the supernatant sera pipetted off.

### *Passage Experiments*

#### *(a) Attempt at passage of serum antibody*

(1) Serum from affected and control animals, respectively, was injected into the exposed jugular veins of normal control animals. By previous experiment it was found that the size of guinea pigs used could stand up to 5 ccs thus injected at one time. The animals were injected twice weekly, and a skin test

was carried out 24 hours after each injection. When an attempt at intravenous injection failed, the serum was injected intraperitoneally so that in all cases the animal received 10 ccs weekly.

(2) In accordance with the technique described by Chase (18) serum from the affected animals and controls was injected intradermally into a normal guinea pig and 0.1 cc of 10% brain emulsion injected into the site 24 hours later, in order to attempt to elicit a Prausnitz-Kustner reaction.

(b) *Attempt at Passage of Cellular Hypersensitivity* The methods used were those described by Chase (19) and consisted of

(1) Sick animals, or animals anticipated to be so, were injected intraperitoneally with 20 ccs of sterile liquid paraffin and killed about 48 hours later. The peritoneal cavities were washed out with 20 ccs heparinized Tyrode solution containing  $\frac{1}{10}$  volume normal guinea pig serum. The cells were separated from the supernatant fluid after standing, and centrifuged. The sediment was washed in heparinized Tyrode solution, and again spun down, and this procedure was repeated two further times, the final sediment being made up to a 3 ccs suspension with heparinized Tyrode containing  $\frac{1}{10}$  vol normal guinea pig serum. The 3 ccs suspension was injected intraperitoneally into a test animal, skin tests were carried 24 hours later.

(2) The spleen and a number of lymph nodes from two exsanguinated animals which had shown paralysis, were ground up together and treated with heparinized Tyrode solution as described for the cellular portion of the peritoneal exudates in (1) above. Five ccs of the suspension were injected intraperitoneally into a test animal, which was skin tested after 24 hours.

(c) *Attempt to Passage Encephalitic Agent* Two brains weighing about 5 gms after thin slices had been removed for histological examination, from affected and exsanguinated animals were ground up in 10 ccs saline and injected intraperitoneally into two normal guinea pigs which were skin tested 24 hours later.

#### CLINICAL SIGNS OF ENCEPHALITIS

*Guinea Pigs* (see Table III)

(a) *Experiment I* Ten out of twelve guinea pigs injected with brain antigen and adjuvant showed signs referable to the CNS. The onset



varied from 14 to 28 days after the injection, and in all cases consisted of weakness of the back and hind legs, in addition three cases showed paralysis of the forelegs, two cases marked ataxia, and two cases showed twitching of limbs. As a rule the onset was fairly sudden and the animal became almost moribund within 24 hours, when it was killed. In two cases (Nos 8 and 10) each animal was allowed to live 5 days, during which time the symptoms became progressively worse.

Two animals (Nos 4 and 9) showed no signs. No 4 was killed on the second day because of a limp (misinterpreted as a paralysis), which was afterwards found to be due to a cervical abscess at the site of inoculation. No 9 was sacrificed on the 42nd day. None of the 12 controls injected with adjuvant mixture showed any signs of CNS involvement, and all were sacrificed on the 42nd day.

(b) *Experiment III* Fourteen out of twenty test guinea pigs showed symptoms referable to the CNS, all of these showed weakness of hind legs and back and two showed ataxia. The earliest case showed the onset of signs on the 11th day, and the latest on the 21st day. All animals were killed within 24 hours of the onset of the symptoms. Six out of twenty test animals died without symptoms within a period of 14 to 25 days and though no cause for death was found grossly at autopsy, four of the animals were found to have had encephalitis on microscopic examination. The cause of death of the remaining two was not determined. Ten control animals injected with adjuvant mixture only, showed no signs and eight were sacrificed on the 35th day. Two died on the 7th and 20th day respectively but no cause for these deaths could be found.

### *Rabbits*

(a) *Experiment II* None of the ten test nor ten control animals showed any signs of CNS involvement, and all were sacrificed at about the 10-11th week.

(b) *Experiment IV* None of the ten test nor five control animals showed any signs of CNS involvement and all were sacrificed at about the 10th to the 12th week, except test animal No 45 which died on the third day from pneumonia and empyema.

(c) *Experiment V* None of the three control nor six test animals showed signs of CNS involvement except test animal No 46 which

showed some weakness of the hind legs on the 11th day following injection. It was killed 24 hours later. The other animals were sacrificed at the 10th to 12th week.

#### SKIN TESTS

*Guinea Pigs* (see Table III) Skin tests were performed on the animals of Experiments I and III, as described under "Methods" on the 14th day following injection. All tests were negative at the site of injection of brain suspension alone. As was expected, however, the same animals showed a delayed inflammatory necrotizing reaction at the site of the injection of the adjuvant mixture which contained dead tubercle bacilli.

*Rabbits* In Experiments III, IV and V skin tests with brain suspensions alone were negative. An inflammatory delayed necrotizing reaction occurred at the site of the injection of the adjuvant mixture containing dead tubercle bacilli and many of these reactions became indolent ulcers which healed very slowly.

#### GROSS AUTOPSY FINDINGS

No gross lesions were found in the CNS of either guinea pigs or rabbits.

In all cases in test and control animals a considerable induration measuring up to 1-2 cm. in diameter was found in the muchal subcutaneous tissues at the site of injection of the brain adjuvant emulsion. Very often these areas were cystic and loculated, and contained a creamy material varying in appearances from that resembling the original injection emulsion to a greenish pus.

#### HISTOLOGY

##### *Guinea Pigs*

In both experiments I and III the positive findings were the same, consisting of acute to subacute focal, perivascular inflammation of the meninges, brain and stem, cerebellum, cord and nerve roots.

In Experiment I, ten out of the twelve test animals showed these lesions. In Experiment III, seventeen out of twenty, none of the twelve and ten controls respectively, in either experiment showed any pathological lesion.

*Brain* This showed a focal encephalitis, which always appeared to be related to the blood vessels (Fig 1) The lesions were situated in both white and grey matter, but the areas most heavily affected were the para-ventricular and subependymal tissues (Fig 4) and the subpial layers of the cortex in relation to the penetrating vessels (Fig 12)

In the acute phases there was intramural and perivascular infiltration of the vessel wall, mainly with migrating monocytes and polymorphonuclear cells which wandered out into the interstitial tissues (Figs 2, 3, 4, 5) In H & E sections the superficial appearances of such an acute inflammatory condition affecting the vessel wall suggested that an acute necrotization had taken place (Fig 2) Closer examination and special stains, however, showed that the wall of the vessel was swollen with an excessive outpouring of inflammatory exudate which had separated the collagen fibres without destroying the continuity of the wall In no cases was it possible to demonstrate a fibrinoid necrosis by special stains Eosinophiles were rarely found Endothelial swelling was marked, and was sometimes associated with thrombosis or blockage of the lumen by leucocytic thrombi (Fig 3)

Haemorrhages were frequent and appeared as small isolated foci in the interstitial tissues around capillaries, or intramurally and perivascularly into the VR spaces of the somewhat larger vessels (Figs 3, 4, 5)

In the less acute lesions the infiltrating cells were mainly lymphocytes, monocytes and epithelioid cells, with only a few polymorphonuclear cells occasionally In some cases the vessels showed a perivascular cuffing or lymphocytes or monocytes (Figs 7, 10) and in others monocytic and epithelioid cells were grouped around the vessels in masses that had the appearance of a granulomatous nodule, sometimes obscuring the lumen (Fig 6) Giant cells were never demonstrated Affected vessels in the deeper parenchymatous tissues had a circumscribed nodular appearance with no apparent reaction in the surrounding tissues

However, beneath the pia and especially beneath the ependyma there was a heavy increase of cellularity of the surrounding tissues due not only to infiltration of monocytic and lymphocytic cells, but also due to some microglial proliferation In these latter areas proliferating astrocytes could be occasionally demonstrated

Fat-laden gitter cells were never demonstrated, and exceedingly

rarely there was an apparent demyelination in a very narrow zone surrounding an acutely inflamed vessel (Fig 8) In the centres of inflammatory foci some chromatolysis of ganglion cells was occasionally seen, but generally the nerve cell bodies showed no degeneration

The tela chorioidea in all cases was focally thickened by a mononuclear cell infiltration and generally the choroid plexus was also involved (23 out of 27 cases affected) (Fig 9)

As far as could be ascertained the above lesions were always related to blood vessels, but it was often difficult to determine the type of vessel involved, arterial or venous, because of the profound inflammatory changes The impression was gained, however, that the venules and capillaries were the main vessels affected, although in some areas all vessels including arterioles were involved Both acute and subacute lesions were often present in the same brain The lesions were most prominent in the cerebrum and present to a conspicuous, but less degree, in the brain stem (especially around the fourth ventricle) In one case, the lesions were found in the stem only

*Cerebellum* Perivascular infiltration by mononuclear cells, but no polymorphonuclear cells, was encountered in nine out of twenty-seven cases affected (Fig 10) but in only one or two was there evidence of slight localized degeneration of Purkinje cells

*Spinal Cord* The lesions were demonstrated in the cord in sixteen out of twenty-seven cases affected Generally they were much milder than those seen in the brain, and in some cases were found only in the upper part of the cord The grey matter was seldom involved

*Nerve Roots* A radiculitis, involving the nerve roots arising from the cord (Fig 11) was demonstrated in eight out of the twenty-seven cases with lesions elsewhere

*Meninges* These showed a focal leptomeningitis which consisted of perivascular infiltrates of mononuclear cells, lymphocytes, plasma cells, monocytes, and sometimes epithelioid cells, affecting heavily the veins and venules (Figs 12, 13) This focal cellular infiltration extended over the cortex, the base (where it was most severe passing up into the choroidal fissure), the stem and only occasionally did it extend down over the spinal cord Polymorphonuclear cells were only rarely present amongst the infiltrating cells of the meninges

*Viscera* No lesions were found in the viscera or sciatic nerves of

any of the test or control animals. At the site of the original nuchal injection a granulomatous scleroma adiposum was found subcutaneously in all animals.

### *Rabbits*

Rabbits 1 and 3 (experiment II) showed a moderate degree of focal meningo-encephalitis. The meninges were focally infiltrated with lymphocytes and the inflammatory process appeared to be extending down the penetrating vessels to the cortical subpial layer. Within the brain there were many circumscribed areas made up of intramural and perivascular infiltrates of lymphocytes, monocytes and epithelioid cells. There was little migration into the surrounding tissues and no neuroglial cell reaction. No lesions were demonstrated in the nervous systems of the other eight test animals and the controls of Experiment II, and no lesions in any test or control animal of Experiments IV and V, except control 74 (Exp. V) which shows a small area of perivascular inflammation (mononuclear cells) in both the pons and cerebrum respectively. There were no visceral lesions demonstrated in any animal other than Rabbit 45 (Experiment IV) which died from a pneumonia, empyema, and focal hepatic necrosis. *Note* Rabbits 1 and 3 of Exp. 11 were housed in a separate laboratory from the other rabbits of the Experiment.

### SEROLOGY

#### *Guinea Pigs* (See Table I and III)

(a) *Experiment I* Of the ten test sera examined, six showed specific complement fixing antibodies to homologous brain antigen. Non-specific antibody reacting with kidney antigen was found in three out of ten sera tested. Neither specific nor non-specific complement fixing antibodies were demonstrable in the sera of twelve control animals nor in the sera of four untreated guinea pigs. No flocculating antibodies to homologous brain or kidney were demonstrated in test, control or normal sera.

(b) *Experiment III* Of the thirteen test sera examined, six showed specific antibodies to homologous brain antigen. Two of these also had non-specific antibody to homologous kidney. One case out of the remaining seven tested had non-specific antibody to kidney only, and another was anticomplementary. Eight out of ten sera from the con-

TABLE I

*Fig. 1, of the Quantitative Serological Tests on the Sera of Guinea Pigs in Experiments I and III, Injected with Homologous Brain and Adjuvant*

EXPERIMENT	CUMULATIVE SERIAL NO.	DAY OF DEATH	COMPLEMENT FIXATION TEST				FLOCCULATION TEST	
			Against brain antigen	Against kidney antigen	Absorbed by brain, against brain antigen	Absorbed by kidney, against brain antigen	Against brain antigen	Against kidney antigen
I	1	22	1 160	0	0	1 160	0	0
	2	15	Not done		—	—	—	—
	3	28	1 120	1 40	0	1 40	0	0
	4	24	1 80	0	0	1 80	0	0
	5	17	1 40	1 10	0	1 20	0	0
	6	22	1 20	1 10	0	1 10	0	0
	7	17	Not done		—	—	—	—
	8	19	0	0	—	—	0	0
	9	42	0	0	—	—	0	0
	10	20	1 10	0	0	0	0	0
	11	14	1 40	0	0	1 20	0	0
	12	17	1 10	0	0	0	0	0
III	43	22	anti complement		—	—	0	0
	40	22	1 10	1 10	0	0	0	0
	41	25	Not done		—	—	—	—
	50	20	Not done		—	—	—	—
	66	14	1 20	0	0	1 20	0	0
	64	15	Not done		—	—	—	—
	75	15	Not done		—	—	—	—
	68	12	0	0	—	—	0	0
	70	16	0	0	—	—	0	0
	71	14	1 20	1 10	0	1 10	0	0
	67	16	1 40	1 10	0	1 30	0	0
	39	16	0	0	0	0	0	0
	65	22	Not done		—	—	—	—
	29	16	0	0	—	—	0	0
	36	19	1 20	0	0	1 20	0	0
	32	21	1 30	0	0	1 20	0	0
	38	11	1 40	0	—	1 40	0	0
	33	18	0	0	—	—	0	0
	73	14	Not done		—	—	0	0
	34	20	Not done		—	—	0	0

12 control sera of Experiment I, and 10 of Experiment III contained no demonstrable specific brain antibodies. Serum for serology was collected in all cases at the time of death.

trol animals were tested, and six of these contained no demonstrable complement fixing antibodies and two were anticomplementary. None of the sera tested contained demonstrable flocculating antibodies.

### *Rabbits* (See Table II)

Complement fixing antibodies to homologous brain antigen were found in all test, control and normal rabbit sera tested. As these positive results were probably due to natural antibody, the sera were heated to 65° for 30 minutes as described by Kidd and Friedenwald (26). After this procedure no specific antibody to brain antigen could be demonstrated in control and normal sera. The remarks below refer to the results of tests carried out after heating.

(a) *Experiment II* Ten out of ten test animals showed specific complement fixing antibodies to homologous brain antigen and there was no demonstrable non-specific antibody reacting with homologous kidney antigen. Ten out of the ten controls and four out of four normal animals showed no demonstrable antibody to homologous brain although one control serum (No. 12) contained non-specific antibody to rabbit kidney antigen. Nine out of ten test sera examined contained specific flocculating antibody to homologous brain antigen and nine out of ten controls and four out of four normal sera were negative. The tenth control serum contained non-specific antibodies to rabbit kidney.

(b) *Experiments IV and V* Seven out of nine test sera tested in Exp. IV showed the presence of specific complement fixing antibody to brain, one was negative and a second anticomplementary. In Exp. V six out of six of the test sera examined contained specific complement fixing antibodies. None of the controls in either experiment (5 and 3 animals respectively) contained specific antibodies, but one case (No. 29) contained non-specific antibody to kidney. In the flocculation experiments similar results were obtained, namely, in Exp. IV seven out of nine and in Exp. V six out of six test sera contained specific antibodies whereas all controls were negative.

### PASSAGE EXPERIMENTS

(a) *Attempts at Passage of Serum Antibody* (See Table IV)

(i) In Experiment I, one guinea pig (No. 131) received 20 ccs during a period of 10 days, of pooled serum. This serum was obtained from

TABLE II

*Results of the Quantitative Serological Tests on the Sera of Rabbits in Experiments II, IV and V, Injected with Homologous Brain (or Cord) Antigen and Adjuvant*

EXP NO	RABBIT SERIAL NO	COMPLEMENT FIXATION TEST				FLOCCULATION TEST			
		Against brain antigen	Against kidney antigen	Absorbed by brain, against brain antigen	Absorbed by kidney, against brain antigen	Against brain antigen	Against kidney antigen	Absorbed by brain, against brain antigen	Absorbed by kidney, against brain antigen
II	1	1 30	0	0	1 30	1 6	0	0	1 6
	3	1 160	0	0	1 160	1 48	0	0	1 48
	5	1 30	0	0	1 30	1 1	0	0	1 1
	7	1 30	0	0	1 30	1 2	0	0	0
	9	1 30	0	0	1 30	1 4	0	0	1 1
	11	1 160	0	0	1 160	1 48	0	0	1 48
	15	1 40	0	0	1 40	1 2	0	0	1 2
	13	1 160	0	0	1 160	1 16	0	0	1 16
	17	1 160	0	0	1 160	1 16	0	0	1 16
	19	1 320	0	0	1 320	1 64	0	0	1 64
IV	36	1 10	0	0	1 10	1 2	0	0	1 2
	37	1 80	0	0	1 80	1 1	0	0	1 1
	38	0	0	0	0	0	0	0	0
	39	1 160	0	0	1 160	1 48	0	0	1 48
	40	1 30	0	0	1 30	1 2	0	0	1 2
	41	1 40	0	0	1 10	1 1	0	0	1 1
	42	1 80	0	0	1 80	1 8	0	0	1 8
	43	Anti complementary			—	1 1	0	0	1 1
	44	1 80	0	0	1 80	1 1	0	0	1 1
	45	not done			—	—	—	—	—
V	46	1 160	0	0	1 160	1 20	0	0	1 20
	47	1 160	0	0	1 160	1 20	0	0	1 20
	48	1 160	0	0	1 160	1 8	0	0	1 8
	84	1 80	0	0	1 80	1 8	0	0	1 8
	21	1 160	0	0	1 80	1 16	0	0	1 16
	49	1 160	0	0	1 160	1 20	0	0	1 20

Ten controls in Experiments II, five in Experiment IV, and three in Experiment V contained natural antibody to brain antigen. This antibody was removed by heating to 65° for 30 minutes and subsequently no specific anti-brain antibody was found in any control in any experiment. Serum for serology was collected in all cases at the time of death.

the six animals which had demonstrable complement fixing antibodies in their blood, following injection of brain antigen, five of these animals showed histological lesions of the brain.



TABLE III

*Summary of the Effects of the Injection of Homologous Brain and Adjuvant into Guinea Pigs of Experiments I and III*

EXPER NO	SERIAL NO	WT IN GMS	SIGNS AND DAY OF ONSET	MODE AND DAY OF DEATH	SKIN TEST 14TH DAY	SPECIFIC ANTIBRAIN ANTIBODY		HISTOLOGIC LESIONS OF CNS
						CF	Floc	
I	1	380	P 22	K 22	0	+	0	+
	2	400	A & P 14	K 15	0	—	—	+
	3	475	P 28	K 28	0	+	0	+
	4	390	0	K 24	0	+	0	0
	5	420	T & P 17	K 17	0	+	0	+
	6	440	P 22	K 22	0	+	0	+
	7	425	A & P 17	K 17	0	—	—	+
	8	475	P 14	K 19	0	0	0	+
	9	425	0	K 42	0	0	0	0
	10	400	P 15	K 20	0	0	0	+
	11	490	P & T 14	K 14	0	+	0	+
	12	375	P 17	K 17	0	0	0	+
III	43	300	A & P 22	K 22	0	Anti-comp	0	+
	40	300	P 22	K 22	0	0	0	0
	41	300	0	D 25	0	—	—	+
	50	305	0	D 20	0	—	—	+
	66	325	P 14	K 14	—	+	0	+
	64	275	0	D 15	0	—	—	+
	75	300	0	D 15	0	—	—	0
	68	300	P 12	K 12	—	0	0	+
	70	315	P 16	K 16	0	0	0	+
	71	325	P & T 14	K 14	—	+	0	+
	67	325	P 16	K 16	0	+	0	+
	39	275	P 16	K 16	0	0	0	+
	65	275	P 22	K 22	0	—	—	+
	29	320	A & P 16	K 16	0	0	0	+
	36	320	P 19	K 19	0	+	0	+
	32	320	P 21	K 21	0	+	0	+
	38	280	P 11	K 11	—	+	0	+
	33	310	P 18	K 18	0	0	0	+
	73	325	0	D 14	—	—	—	0
	34	325	0	D 20	0	—	—	+

22 Control animals in both experiments gave no reactions clinically, were negative to skin tests, complement fixation or flocculation tests and were negative histologically. Serum for serology was collected in all cases at time of death.

A—ataxia, T—tremors, D—Died, P—paralysis, K—killed

In Experiment III, two animals (Nos 80 and 96) received 50 ccs and 30 ccs of pooled serum during periods of 35 and 21 days respectively. This serum was obtained from thirteen animals, six of which contained demonstrable antibody in their blood and twelve of which

TABLE IV

*Attempt at Passage of Antibody from Affected Guinea Pigs in Experiments I and III*

EXP NO	POOLED SERA USED	MODE OF INJECTION	TOTAL AMOUNT OF SERA INJECTED	TOTAL PERIOD OF INJECTION (DAYS)	SERIAL NO OF NORMAL GUINEA PIG INJECT	SKIN TESTS 24 HR AFTER INJECT	CLINICAL SIGNS	MODE OF DEATH & DAY FOLLOWING LAST INJECT	PROCT. LESIONS	HISTOLOGY
I			cc							
	Test	IV	20	10	131	0	0	K 31	0	0
	Test	IV	5	4	132	0	—	D 2nd inject	0	0
	Control	IV	5	4	133	0	0	D 4	0	0
	Control	IV	20	10	134	0	0	K 31	0	0
III	Test	IV & IP	50	35	80	0	0	K 56	0	0
	Test	IV & IP	30	21	96	0	0	D 21	0	0
	Control	IV & IP	50	35	92	0	0	K 56	0	0
	Control	IV & IP	15	11	42	0	0	D 12	0	0

IV and IP = Intravenous and intraperitoneal

K = killed

D = died

showed histological lesions of the brain, following injection of brain antigen. Only six out of the thirteen animals which contributed to the pool, had both circulating antibody and histological lesions at the same time.

None of these three animals showed any reaction clinically by skin test. Autopsy and histology were negative. Controls were all negative.

(ii) Ten sera from test animals of Experiment I, and thirteen sera

from test animals of Experiment III, were tested, along with control sera, by intradermal (Prausnitz-Kustner reaction) injection with no positive result. Six of the sera from the test animals of each experiment contained demonstrable antibody.

TABLE V

*Attempt at Passage of Cellular Hypersensitivity from Affected Guinea Pigs in Experiment III*

SERIAL NO OF TEST ANIMALS USED	MATERIAL USED	SERIAL NO OF NORMAL GUINEA PIG INJECT	SKIN TEST 24 HRS	CLIN SIGNS	SPECIFIC ANTIBRAIN ANTIBODY		MODE OF DEATH AND DAY	GROSS LESIONS	HIS TOLOGY
					CF	Floc			
67	Spleen and lymph nodes	582	0	Ill 19th day	Ant	0	K 19	0	0
39		549	0	0	com 0	0	K 42	0	0
67	Cerebrum	519	0	0	—	—	D 5	0	0
39		98	0	0	0	0	K 42	0	0
43	Periton	575	0	0	—	—	D 4	—	—
65	Washings	590	0	P 19	0	0	K 19	0	0
36	After I P	466	0	0	0	0	K 35	0	0
32	Paraffin	94	0	0	0	0	K 35	0	0

K = Killed

D = Died

P = Paralysis

All the normal animals were injected intraperitoneally with the cellular tissue from affected animals. Serum for serology was collected in all cases at the time of death.

(b) *Attempt at Passage of Cellular Hypersensitivity* (Table V)

(i) Guinea pigs Nos 65, 36, 32, 43 were injected intraperitoneally with sterile liquid paraffin.

No 36 received the paraffin 16 days after the original nuchal injection of brain tissue and showed paralysis three days later and was exsanguinated and killed and the peritoneal washings injected into guinea pig No 46, as described under "Methods". This animal showed no effect from the injection, gave a negative skin test, negative serology at the time of death and showed no gross or histological lesions.

No 32 was also given the paraffin injection 16 days following the original injection and showed paralysis five days later and was killed.

The peritoneal washings were injected into guinea pig No 94 which was sacrificed after 35 days and showed no clinical or skin test reactions, negative serology at the time of death, and negative autopsy and histological findings

No 65 received the paraffin intraperitoneally eighteen days after the original injection and four days later became paralyzed and was sacrificed. The peritoneal washings were injected into No 590 which gave a negative skin reaction after 24 hours. Nineteen days later the animal showed some weakness of the back and right hind leg and paralysis of the left hind leg. It was exsanguinated and killed, serology at the time of death was negative and at autopsy the CNS and viscera were also negative and no cause for the illness was demonstrated histologically.

No 43 also received the paraffin 18 days after the original injection, and showed paralysis after 4 days when it was killed. The peritoneal washings were injected into guinea pig No 75 which gave no reaction clinically and none to skin test, after 24 hours. The animal died after 4 days and no cause was found either at autopsy nor in subsequent histological examination.

(ii) Guinea pigs Nos 67 and 39 were killed on the 16th day following the injection of brain tissue, and emulsions were made from the spleen and lymph nodes. These were injected into guinea pigs 582 and 549. Both of these latter animals gave negative skin tests. No 582 became ill after 19 days and was killed. No lesions were found at autopsy, grossly or histologically. The serum at the time of death was anti-complementary and the flocculation test was negative. No 549 remained well for 6 weeks when it was sacrificed. Autopsy and histology were negative and serological tests showed no demonstrable antibodies at the time of death.

#### DISCUSSION

In the two experiments on guinea pigs the first objective of these experiments was attained, namely, an encephalitis was produced in 27 out of 32 animals injected by a single subcutaneous injection of a saline suspension of homologous brain emulsified with a water-in-oil suspension of tubercle bacilli and an absorption base (Falba). Phenolized

and heated suspensions did not differ appreciably in effect from fresh, untreated tissue suspensions (17). Twenty-four of the thirty-two test animals showed signs of paralysis, and in some cases ataxia and tremors. In the majority of cases the signs occurred within the third week, the earliest being on the 11th day and the latest on the 28th. Such an early onset was also found by Freund et al (1) in their experiments on guinea pigs.

Twenty-three out of the twenty-six cases exhibiting paralysis showed lesions of the brain histologically. The occurrence of paralysis in the absence of demonstrable lesions of the brain such as occurred in No. 40 is not an unknown phenomenon following the injection of brain tissue into the experimental animal, and has been well discussed by Hurst (3). It is, of course, possible that more sections of the brain would reveal lesions in such cases.

In addition, six animals in Experiment III died overnight and were not observed to have had signs referable to the CNS. Four of these animals had brain lesions microscopically. Two animals in Experiment I definitely had no clinical signs and no microscopic lesions in the sections that were examined.

Twenty-seven out of thirty-two test animals showed lesions of the CNS, and the main reaction appeared to be a vascular and perivascular inflammation varying from an acute to a subacute stage (longest duration was 28 days), both stages being present in the same brain, indicating that the process was a continuing one. These lesions seemed to be entirely localized to the CNS for a careful search of other organs revealed no other lesions.

In the H and E sections the acute lesions resembled, superficially, those of periarteritis nodosa, but more careful study showed no necrotization of the vessel walls. The main infiltrating cell was the monocyte, although the polymorphonuclears were present in large numbers. Contrary to the description of similar lesions in monkeys by Morgan (10), eosinophils were rarely found. There was often a heavy outpouring of exudate in the Virchow-Robin space and the latter often contained haemorrhage. Pericapillary haemorrhages in the interstitial tissues were common. Associated with the acute lesions there were sometimes minute thrombi, but little perivascular parenchymatous damage of ganglion cells or demyelination was noted. Necrosis of

brain tissue was never encountered. Microglial proliferation was not a marked feature.

It is true that an apparent demyelination was demonstrated here and there in a narrow zone around a vessel acutely affected, but none was demonstrated in the interstitial tissues at a distance from the vessels. Further, the absence of fat-laden gitter cells makes it probable that the apparent demyelination seen in the special stains was due to the superimposition of an acute inflammatory exudate on the perivascular tissues. It was noted in the sections stained for myelin that the guinea pig brain appears to have less myelinated tissue compared with other animals. This may be one of the reasons why demyelination was not obvious in these experiments and why Freund et al (1) report the complete absence of demyelination in their similar series of experiments with guinea pigs. The latter investigators suggest that the lack of demyelination, which is found in other species affected by an experimental encephalitis (2), (9), (10), may be due to a difference in species.

In the less acute lesions the infiltrates were mononuclear, varying from a thin lymphocytic perivascular cuffing to a florid granulomatous looking mass. Such lesions have been described previously in experimental encephalitis induced by the injection of brain tissue (5), (9), (10), (23). Freund et al (1) in their guinea pigs describe lesions essentially similar to these. No giant cells were encountered, and they were absent in the histological findings of others who have used the relatively rapid adjuvant technique (1) (2) (10). Earlier workers who have used the prolonged injection technique (5), (6) found giant cells conspicuous in the brain lesions, as did Morrison (2), using the adjuvant technique in rabbits, in one animal surviving 92 days.

Two animals (Nos. 8 and 10) allowed to live for five days after the onset of paralysis showed no different histological appearances from others which were killed on the day of onset of neurological signs. Neither showed circulating complement fixing antibody at the time of death.

All meningeal infiltrates were mononuclear, with polymorphonuclears almost conspicuous by their absence. Freund et al (1) found that the choroid plexus and cerebellum were unaffected in their series of cases. This was not the case in this series of experiments for the

choroid plexus, especially at its base, was often heavily infiltrated, and the cerebellum also affected. Involvement of choroid plexus and cerebellum is also reported by others (5) (9) (23).

In the three experiments on rabbits the first objective of this investigation was not attained, namely, the production of an encephalitis.

In Experiment V in which the work of Morrison (2) was repeated using the fresh cord plus adjuvant emulsion, one animal, No. 46, showed weakness of the hind legs on the 11th day, but subsequent examination disclosed no histological lesions in the sections studied. The neurological signs in this case may possibly have been due to the effects of the injected brain tissue as discussed by Hurst (3). However the reported results by Morrison who produced lesions in 8 cases out of 10 injected rabbits, were not obtained in the 6 rabbits treated in the present study.

In Experiment II, two rabbits showed perivascular mononuclear cell infiltrates without neurological signs. These two animals were separated from the rest and it is possible that these animals were infected by the encephalotoxoon caniculi or some other infectious agent as suggested by Schwentker and Rivers (15) in regard to rabbits with similar lesions in their experiments. It is of interest to note that McCartney (24) found similar lesions in 55% of alleged normal laboratory rabbits.

Regarding the second objective of this investigation, twelve out of twenty-three test guinea pig sera examined showed specific complement fixing anti-brain antibodies at the time of death. The titre was fairly low and seemed to have no exact relationship to the period between the time of the initial infection and the onset of paralysis (Table III).

There also appeared to be no exact correlation between the appearance of paralysis or histological lesions and the presence of circulating complement fixing antibody at the time of death, for in 8 cases which showed clinical and histological signs of encephalitis, no antibodies could be demonstrated. (Two of these were allowed to live 5 days after the onset of paralysis and the other six were sacrificed within 24 hours of the onset.)

One case, No. 46, which was killed by mistake and which had a cervical abscess but no microscopic lesions, possessed a titre of 1:80 against brain antigen.

It could of course be argued that the low titre of antibody or the absence of antibody in some of the cases affected, was due to absorption in the antigen-antibody reaction (if the latter is the mechanism by which the lesions are produced)

Flocculating antibodies were not demonstrated in any of the guinea pig sera

In the rabbit series, all test animals gave specific complement fixing and flocculating antibrain antibodies using saline-brain-suspension as antigen. It is probable that saline-brain-suspension comes under the category of "Wassermann-type substance" which can evoke complement fixing and flocculating antibody formation easily in the rabbits, but not in other animals (16). Schwentker and Rivers (15) were able to produce them in rabbits by the intraperitoneal injection of aqueous brain suspension. It was of interest that all rabbit sera, test, control and normal, contained "natural" complement fixing antibodies to saline-brain-suspension antigen. Rabbit sera is, for reasons unknown, liable to this non-specific deviation of complement (16) (25), but these natural antibodies can be destroyed by heating to 65° for 30 minutes as described by Kidd and Friendenwald (26).

Several workers (11) (12) (13) have shown that brain cortex contains a lipid which can act as a haptene, and that this is capable of uniting with protein to form complement fixing antibodies which tend to be organ specific. Further, Schwentker and Rivers (15) showed that the specific antigenicity of homologous brain runs parallel to the myelin content, being absent in foetal and newly-born brains and six times more abundant in white matter than in grey matter.

Despite these findings it is not yet established, either by previous investigators or by the present study, that circulating antibodies produced by the injection of brain tissue are the cause of the histological lesions in the brain that result from the injection.

The third objective of this investigation, the attempt to produce the encephalitis in previously normal animals by passive transfer of serum or tissue or blood cells from affected animals, was negative in the twelve animals subjected to the tests.

In the attempts at passage of cellular hypersensitivity (Table V) one of the six animals (No. 590) became paralyzed on the 19th day following injection. It had given a negative skin test, and negative



serology at the time of death. No lesions were found in the brain histologically and the cause of the paralysis is not evident.

Skin tests with brain tissue on all test animals were negative and none exhibited an immediate or delayed reaction indicative of a hypersensitive state. Freund et al (1) also obtained negative results in guinea pigs injected with homologous brain.

Rivers and Schwentker (5), injecting affected brain tissue from test animals intracerebrally into normal animals, were unable to demonstrate an infective agent responsible for the encephalitis.

The injection of affected cerebrum interperitoneally into two normal animals in the present study was negative (Table V).

Histologically, the main lesion of the encephalitis was a vascular inflammation associated with a mononuclear cell reaction. The vascular changes may well be secondary to a primary perivascular inflammatory focus where antibody meets antigen. However, the lesions suggest in the acute stage, a primary vascular inflammation. Such a concept would postulate special properties of the cerebral vessels for no other vascular lesions were encountered in the viscera of affected animals.

Finally, much speculation has taken place over the alleged similarity of appearance of the experimentally acute lesions and those of acute haemorrhagic leuco-encephalitis (27) and acute disseminated encephalomyelitis. The more chronic lesions have been compared to those in multiple sclerosis and Schilder's disease. It is, however, fruitless to conjecture on the relationship of experimental disease to human encephalitis until further light is thrown on the mechanism of the production of the lesion in the guinea pig described in these experiments.

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#### SUMMARY

A meningo-encephalomyelitis was produced in twenty-seven out of thirty-two guinea pigs injected subcutaneously with a saline suspension of homologous brain in water-in-oil emulsion of dead tubercle bacilli.

Specific anti-brain complement fixing antibodies were demonstrated

in twelve out of twenty-three test guinea pigs Eight cases showing lesions had no demonstrable antibody The present studies have failed to demonstrate a relationship between the meningo-encephalitis and the presence of antibrain antibodies

Attempts at passive transfer, using sera or living cells, were essentially negative, as were skin tests for hypersensitivity

Attempts at producing an encephalitis in rabbits using methods similar to the above were, in the main, unsuccessful, although unexplained lesions were found in two test animals and one control animal

Specific antibrain complement fixing and flocculating antibodies were produced in rabbits as a result of the injecting of saline-brain-suspension plus adjuvant emulsion

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### ILLUSTRATIONS

FIG 1 G P No 39 Paralysis 16th day, killed on 16th day Low power view of cerebral cortex showing an acute vascular and perivascular inflammatory reaction The infiltrating cells are monocytes and polymorphonuclear cells There is little microglial response

FIG 2 G P No 39 Paralysis 16th day, killed on the 16th day Cerebrum showing an acute vascular inflammation with intramural and perivascular infiltration of monocytes and polymorphonuclear cells The appearances are suggestive of periarteritis nodosa, but though the wall may appear destroyed in the photograph, under the microscope the fibres of the vessel wall are seen to be merely widely separated by exudate and special stains show the continuity of the wall to be intact

FIG 3 G P No 11 Paralysis 14th day, killed on 14th day The pons, showing an acute encephalitis To the left is a large penetrating vessel, the lumen of which is almost occluded by a leucocytic thrombus To the right is a large vessel showing a recent haemorrhage into the V R space Interstitial pericapillary haemorrhages are frequent and are represented by aggregates of black dots

FIG 4 G P No 11 Paralysis 14th day, killed on the 14th day This shows an acute subependymitis in the tissues bordering the lateral ventricle There is a heavy perivascular cuffing with monocytes, lymphocytes and polymorphonuclear cells Most of the cells infiltrating the interstitial tissues are haematogenous but there is some microglial proliferation The central vessel is thrombosed At the left centre (in square) is a vessel showing distension of the V R space with blood clot There are many small interstitial haemorrhages not shown in the photograph

FIG 5 G P No 11 High power view of the vessel depicted in Fig 4 by square Blood clot is distending the V R space

FIG 6 G P No 29 Paralysis 16th day, killed on the 16th day High power view of one of the perivascular granulomatous-like lesions The infiltrating cells are monocytes and epithelioid cells

FIG 7 G P No 11 Paralysis 14th day, killed on the 14th day Two vessels in the cerebrum showing heavy intramural and perivascular infiltrates of mononuclear cells The smaller vessel is occluded A neighbouring ganglion cell has a pyknotic nucleus Figs 3, 4, 5 show an acute inflammatory reaction taking place in the same animal

FIG 8 G P No 39 Paralysis 16th day, killed on 16th day Cerebral cortex showing a narrow zone of apparent demyelination Such appearances as these were of infrequent occurrence No gutter cell response could be demonstrated histologically in this animal

FIG 9 G P No 7 Paralysis 17th day, killed on the 17th day Choroid plexus (lateral ventricle) showing a mononuclear infiltration

FIG 10 G P No 11 Paralysis on 14th day, killed on 14th day Shows a perivascular cuffing of a cerebellar vessel

FIG 11 G P No 36 Paralysis 19th day, killed on the 19th day Nerve root from the cervical cord, showing a radiculitis

FIG 12 G P No 6 Paralysis 22nd day, killed on 22nd day Meninges of the choroidal fissure showing a thickening due to a dense mononuclear cell infiltration Note the penetrating vessel which shows intramural and perivascular mononuclear cell infiltration

FIG 13 G P No 10 Paralysis 15th day, killed on 20th day Shows a mononuclear infiltration of the meninges over the medulla



FIG 1

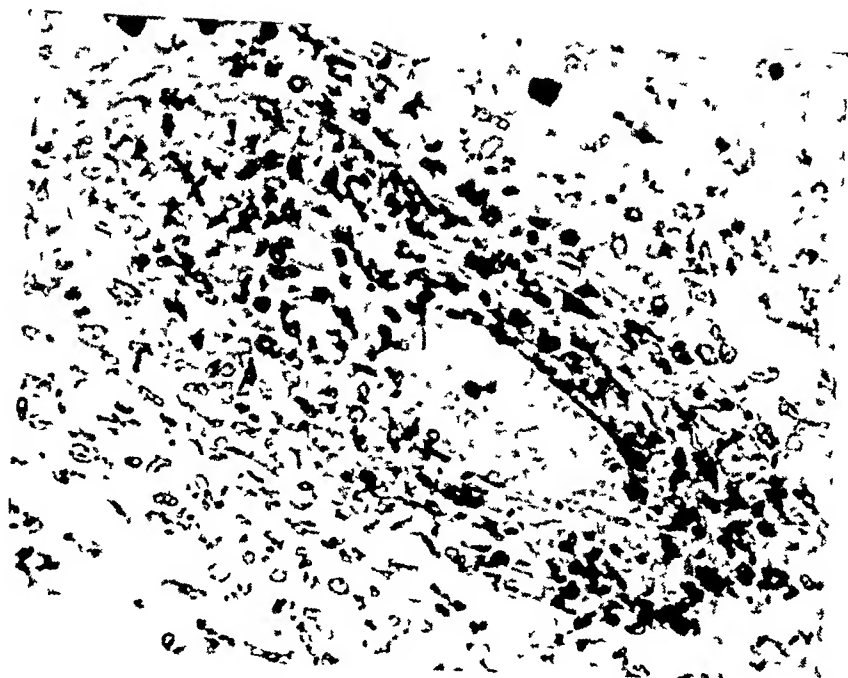


FIG 2



FIG 3

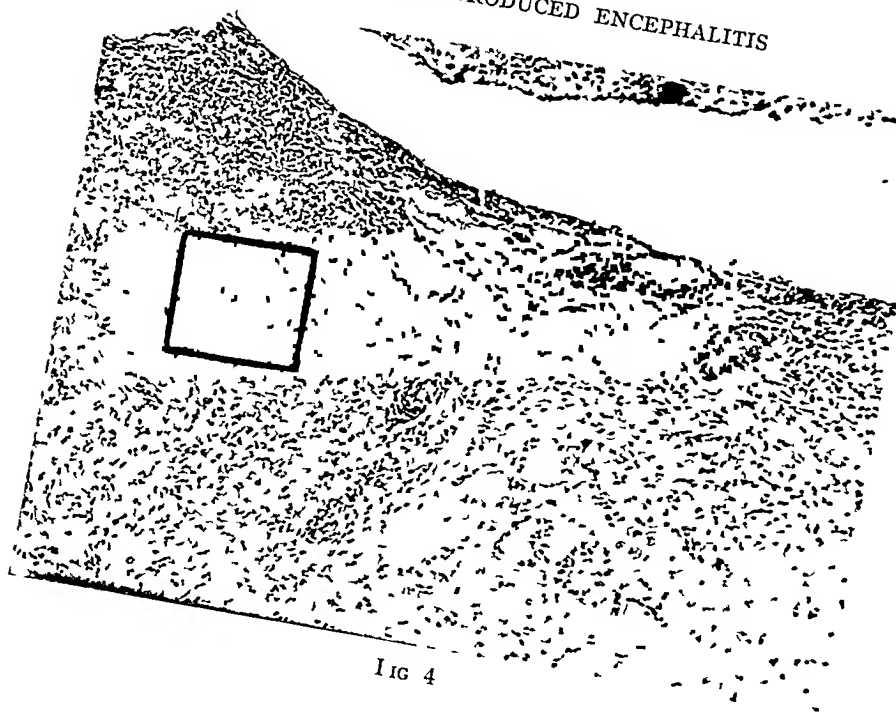


FIG 4

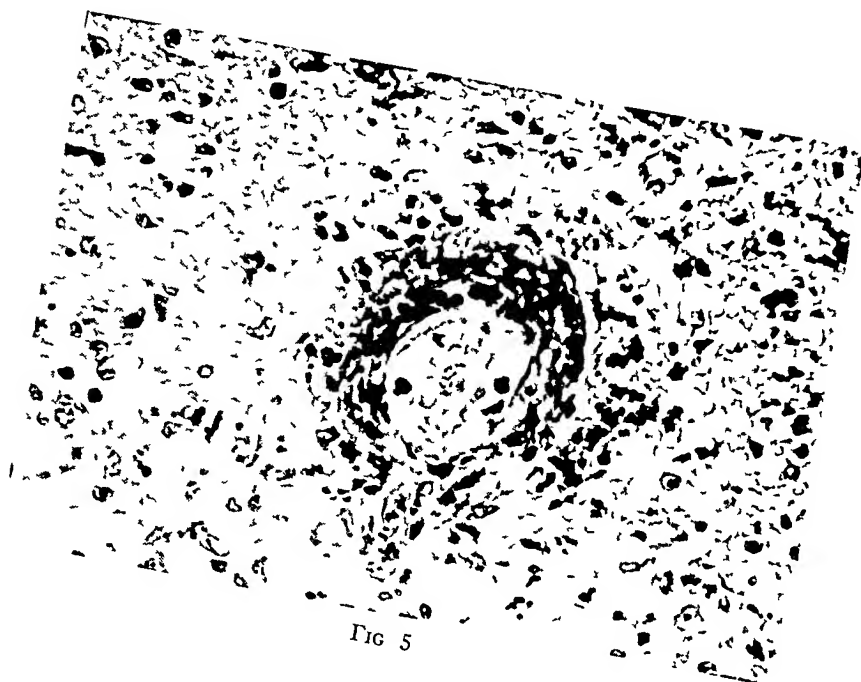


FIG 5

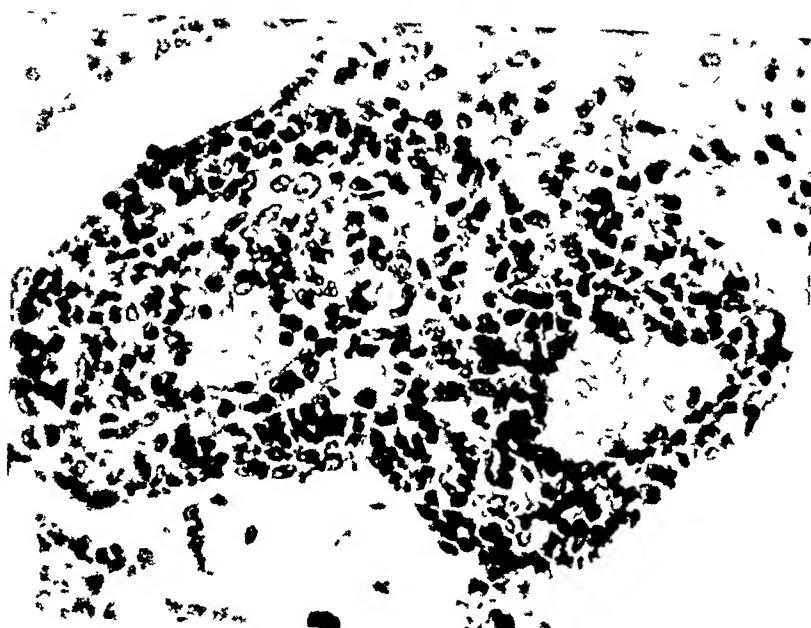


FIG 6

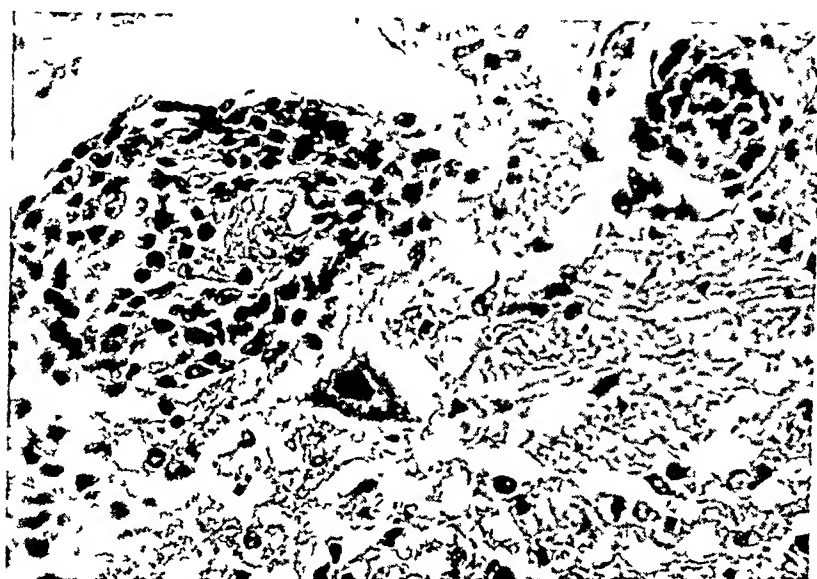


FIG 7

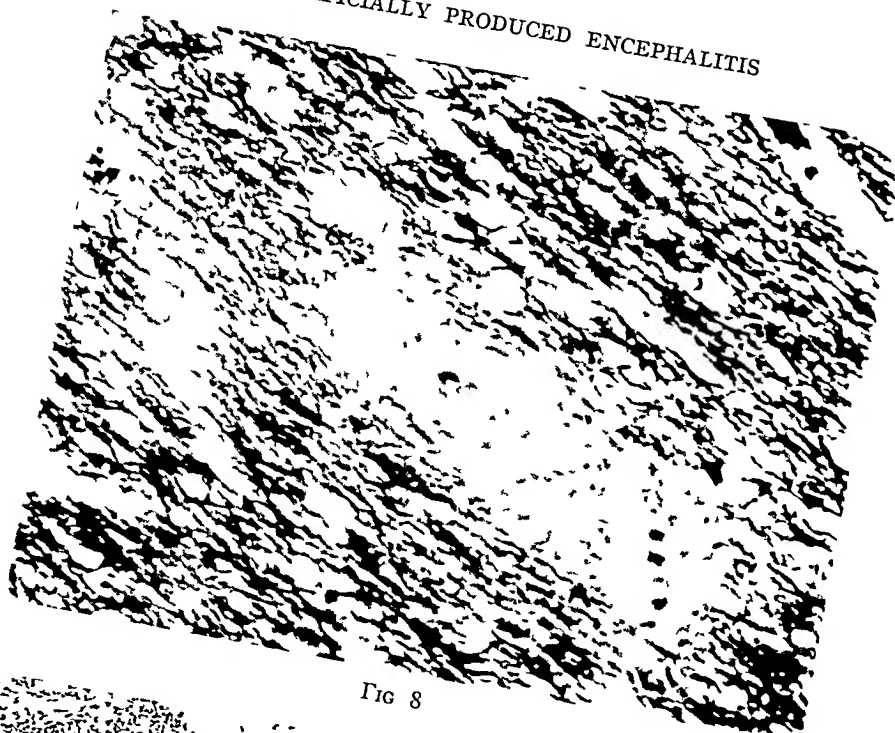


FIG 8

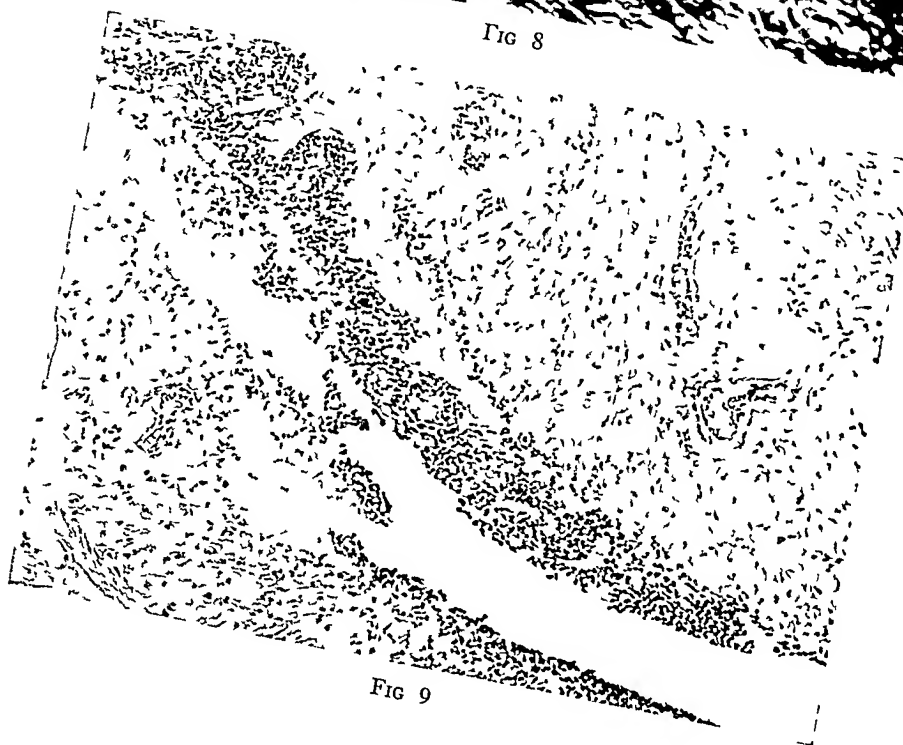


FIG 9





FIG 10



FIG 11

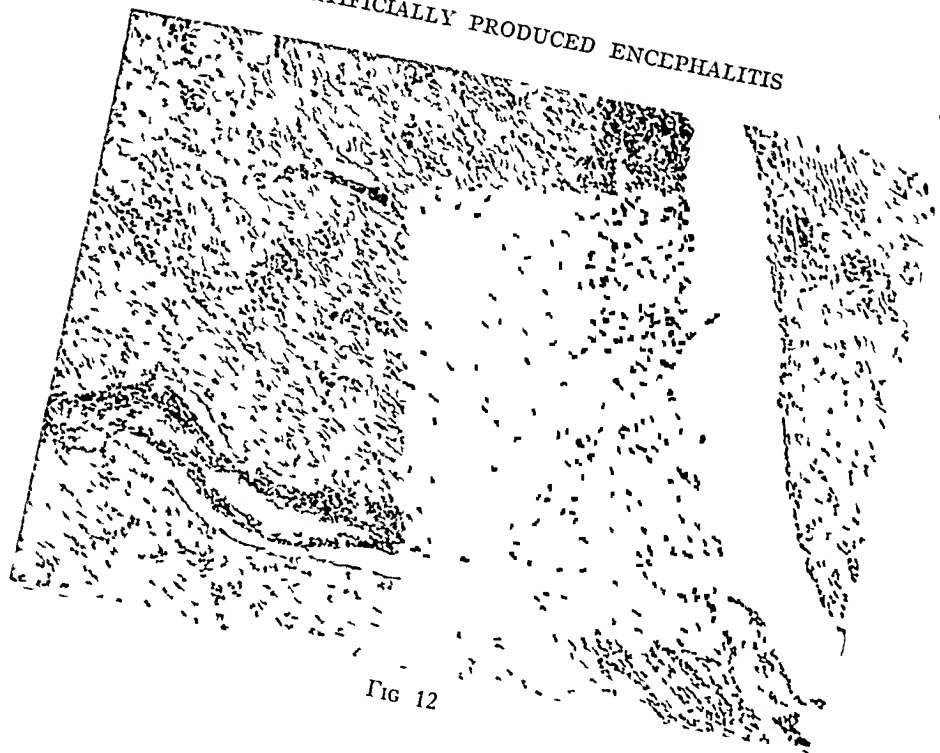


FIG 12

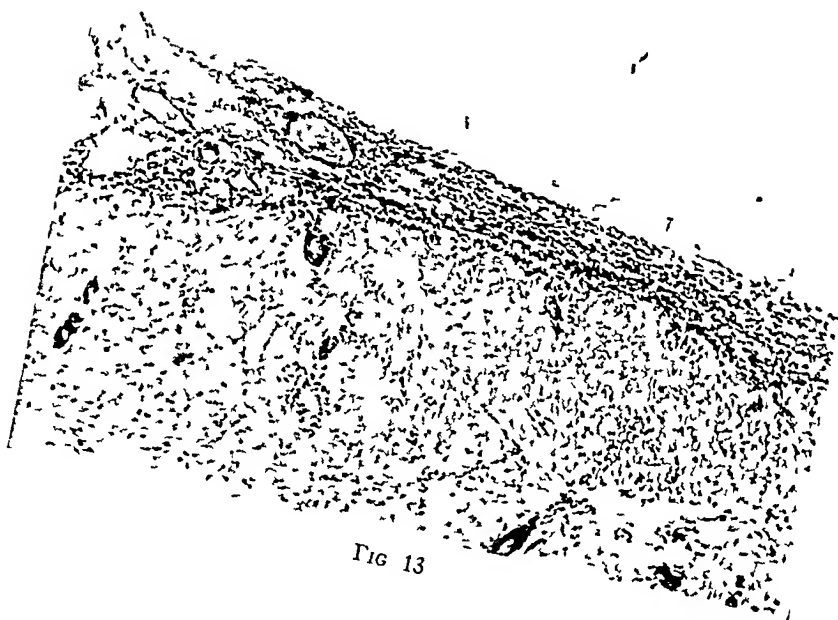


FIG 13

# ANTI-PNEUMOCOCCAL POWERS OF THE BLOOD IN ALLOXAN DIABETES IN THE RABBIT

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## INTRODUCTION

In a previous paper (1) it was pointed out that the method of producing diabetes with alloxan made it possible to investigate, under controlled conditions, the notorious susceptibility of diabetics to infection. In that paper the results of a study of antibody formation were presented, and were that there is no impairment of antibody formation in rabbits with alloxan diabetes. The present paper gives the results of an investigation of phagocytosis under similar conditions.

Quite soon after the suggestion of Lassar in 1904 (2) that it was the increased sugar content of the blood and tissues that favored bacterial growth, evidence began to accumulate that such an explanation of diabetic susceptibility to infection was too simple. Phagocytosis was studied by Da Costa (3) and Da Costa and Beardsley (4) and they found that the opsonic index in patients with diabetes mellitus was low. In 1911 Handmann (5) found that when he added glucose to normal blood so that a concentration similar to that in diabetic blood was produced, the bactericidal power of the blood for staphylococci was not decreased, nor was the growth of the implanted bacteria favored. Similar results were obtained by Hirsch-Kauffman and Heimann-Trosien (6) when they produced hyperglycemia, lipemia, and acidosis artificially. The latter authors found that hyperglycemic blood from diabetic children without acidosis did not cause an increase in the growth of implanted bacteria when compared with normal human blood, but that blood from three comatose patients did so. After the same patient had been treated with insulin and brought out of coma, bacterial growth in their blood was the same as in normal blood. About the same time the effect of insulin upon phagocytosis was studied

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by Bayer and Form (7, 8) and was found to be stimulating to the phagocytic power of normal horse leycocytes in vitro, and to raise the opsonic index of pancreatectomized dogs

In 1933 Richardson (9) reported the results of his studies of specimens from human diabetics and from experimental animals, and stated that "Diabetic whole blood, regardless of the level of its blood sugar, has in general a weaker bactericidal property than has non-diabetic whole blood as tested by standard methods"

In the absence of complete agreement by investigators and because of the lack of clear cut results it seemed to A R Rich (10) that "The matter is of sufficient interest and importance in relation to the problem of resistance to tuberculosis and to other infections to warrant further careful study" At Dr Rich's suggestion the following experiments were undertaken

#### MATERIALS AND METHODS

Male albino rabbits were made diabetic by the intravenous injection of alloxan in 4 per cent solution in saline In most of the animals a single dose of between 100 mgm per kgm and 200 mgm per kgm was used, but in some of the animals which had received the smaller doses a second and sometimes a third dose was required Occasionally an animal could not be made diabetic The animals were not fasted before administration of the alloxan, and glucose and insulin were not used Blood sugar estimations were carried out by the method of Folin and Malmros (11) The specimens were not fasting samples of blood and animals with sugar levels below 300 mgm per cent were not included in the experiment A certain number of rabbits died of diabetes quite soon after having been injected The ones used in these experiments, however, had all survived, without treatment and with continuous hyperglycemia, polyuria and glycosuria, for at least five months None had acidosis Some maintained their weight, others became thin Diabetics and normal controls were fed an unlimited diet of Purina Laboratory Chow and water Polydipsia was marked in the diabetics Some of the diabetic animals had been used in previous experiments in which they had been immunized against crystalline egg albumen The level of their serum proteins was normal

The bactericidal powers of the blood of the diabetics and of normal

TABLE I

	NUMBER OF COLONIES COUNTED IN POURED BLOOD AGAR PLATES INOCULATED WITH 1 C C OF DILUTIONS LISTED				W B C PER CU MM	BLOOD SUGAR	URINE SUGAR	WEIGHT KG	DURATION OF DIABETES	INGESTED COCCI PER POLY- MORPH
	$\frac{1}{50,000}$	$\frac{1}{100,000}$	$\frac{1}{1 \text{ million}}$	$\frac{1}{10 \text{ million}}$						
Mar 6, 10 30 am Rabbit 23 D Rabbit 57 N  6 pm Rabbit 23 D Rabbit 57 N	134 80	55 52	5 4	— —	6,800 8,400	433 90	+++ 0	3 0 4 1	5 —	1 3 3 9
	too many "	too many "	600 311	— —						
Mar 16, 11 30 am Rabbit 6 D Rabbit 55 N  7 pm Rabbit 6 D Rabbit 55 N	120 116	48 45	3 4	0 0	6,600 8,000	390 105	+++ 0	3 85 3 1	12 —	3 2 3 8
	too many "	too many 500	272 80	29 9						
Mar 22, 11 am Rabbit 51 D Rabbit 57 N  6 pm Rabbit 51 D Rabbit 57 N	118 137	77 86	10 10	2 0	6,000 5,940	340 90	++ 0	2 3 4 1	6 —	7 6 4 4
	too many "	too many "	496 196	46 20						
Mar 23, 11 am Rabbit 21 D Rabbit 57 N	168 170	76 69	9 9	0 0	9,000 8,400	393 90	+++ 0	3 3 4 1	5 —	8 5 7 9

[illegible]

D—Diabetic, N—Normal,

controls were tested against a type II pneumococcus, of low virulence and with a small capsule. When heavy growth of this organism occurred in a sample of blood, methaemoglobin production caused brownish-black discoloration of the blood, and this was a valuable naked eye indication of the progress of the experiments to be described.

In each experiment, in which aseptic precautions were observed throughout, 5-6 ccs of blood were collected from an ear vein of a diabetic rabbit in a sterile tube containing 0.1 cc of heparin (Abbott). A similar sample was collected from a normal control, and total white cell counts were done on each. Then 3 ccs from each were measured with a pipette and were placed in Wassermann tubes sealed with rubber bungs. Each of these tubes was inoculated with 0.1 cc of the overnight growth in ascitic fluid broth of the pneumococcus, and the tubes were rotated in the incubator at 37°C for a few minutes to produce mixing. Then a sample of 0.1 cc was withdrawn from each tube and the tubes were then returned to the 37°C incubator, where they were rotated mechanically at the rate of 8 revolutions per minute. Serial dilutions were then made of the samples that had been withdrawn, using peptone water as the diluent, and blood agar pour plates were inoculated with 1 cc each of dilutions of  $\frac{1}{50,000}$ ,  $\frac{1}{100,000}$ ,  $\frac{1}{1,000,000}$ ,

$\frac{1}{10,000,000}$ , of the two samples. The plates were incubated at 37°C for 24 hours and were then stored in the ice box to enhance the zone of hemolysis around the colonies of pneumococci. The colonies were then counted.

The tubes of blood which had been returned to the incubator were rotated until there was an appreciable color difference. As a rule about  $7\frac{1}{2}$  hours of incubation were necessary. During the first 1-1½ hours smears were made and were stained by Wright's method. Then the average number of pneumococci taken up by each polymorph was estimated from a count of the bacteria contained in 100 polymorphs. When a color difference was observed another sample of 0.1 cc of blood was taken from each tube and a second series of dilutions, similar to the first, was made and blood agar pour plates were inoculated with 1 cc of each dilution. These were then incubated, and the colonies were

TABLE II

*Plasma*

	NUMBER OF COLONIES COUNTED IN POURED BLOOD AGAR PLATES INOCULATED WITH 1 C C OF DILUTIONS LISTED				BLOOD SUGAR	URINE SUGAR	WEIGHT KG	DURA- TION OF DIA- BETES
	$\frac{1}{50,000}$	$\frac{1}{100,000}$	$\frac{1}{1 \text{ ml-lion}}$	$\frac{1}{10 \text{ mllion}}$				
								mos
<i>April 2, 11 36 a m</i>								
Rabbit 6 D	120	64	6	0	390	+++	3 85	12
Rabbit 53 N	147	89	11	0	90	0	2 8	—
<i>7 p m</i>								
Rabbit 6 D	too many	too many	62	7				
Rabbit 53 N	" "	" "	581	63				
<i>April 9, 11 30 a m</i>								
Rabbit 9 D	61	24	4	0	433	+++	3 35	12
Rabbit 54 N	51	24	3	0	95	0	3 1	
<i>7 p m</i>								
Rabbit 9 D	too many	too many	386	45				
Rabbit 54 N	" "	" "	928	137				
<i>April 1, 12 noon</i>								
Rabbit 51 D	145	68	8	1	340	++	2 3	6
Rabbit 55 N	169	68	8	0	105	0	3 1	
<i>7 30 p m</i>								
Rabbit 51 D	too many	too many	345	43				
Rabbit 55 N	" "	" "	321	50				
<i>March 30, 12 noon</i>								
Rabbit 28 D	161	86	9	2	320	++	3 2	7
Rabbit 57 N	166	87	15	2	90	0		
<i>7 p m</i>								
Rabbit 28 D	too many	too many	204	27				
Rabbit 57 N	" "	" "	213	28				
<i>April 13, 10 30 a m</i>								
Rabbit 27 D	103	67	4	1	366	+++	2	7
Rabbit 18 N	97	45	5	0		0		
<i>6 30 p m</i>								
Rabbit 27 D	too many	too many	197	20				
Rabbit 18 N	" "	" "	655	78				



TABLE II—*Concluded*

	NUMBER OF COLONIES COUNTED IN POURED BLOOD AGAR PLATES INOCULATED WITH 1 C C OF DILUTIONS LISTED				BLOOD SUGAR	URINE SUGAR	WEIGHT KG	DURA TION OF DIA BETES
	$\frac{1}{50,000}$	$\frac{1}{100,000}$	$\frac{1}{1 \text{ mil lion}}$	$\frac{1}{10 \text{ mil lion}}$				
<i>April 19, 11 30 a m</i>								<i>mos</i>
Rabbit 21 D	77	36	8	1	393	+++	3 3	5
Rabbit 57 N	133	64	9	1	90	0	4 1	
<i>7 p m</i>								
Rabbit 21 D	too many	too many	226	34				
Rabbit 57 N	" "	" "	384	62				
<i>April 20, 11 15 a m</i>								
Rabbit 34 D	75	32	7	0	363	+++	3 05	7
Rabbit 54 N	61	22	2	0	95	0	3 1	
<i>6 30 p m</i>								
Rabbit 34 D	too many	too many	409	47				
Rabbit 54 N	" "	" "	303	35				
<i>April 21, 11 a m</i>								
Rabbit A D	109	42	0	1	450	+++	2 3	20
Rabbit 53 N	87	48	5	0	90	0	2 8	
<i>6 p m</i>								
Rabbit A D	too many	too many	329	45				
Rabbit 53 N	" "	" "	201	19				

D—Diabetic, N—Normal

counted after storage in the cold exactly as was done in the case of the plates made from the samples taken before incubation

## RESULTS

The results of eight experiments, with relevant data about the animals, are summarized in Table I. In the case of the diabetic animals, the blood sugar figure quoted is an average of several estimations during the period of the experiments. In the case of the normal animals only one blood sugar estimation was carried out. On plates from the lower dilutions after incubation there were so many colonies that they

were uncountable. It will be seen from the table that in every case the growth in the diabetic blood after incubation was two or three times as heavy as that in the normal, although the initial bacterial counts were approximately equal.

To test whether the above findings with whole blood were related to the sugar content of the blood, eight experiments were carried out in the same way, except that plasma was used instead of whole blood for, because of the great diffusibility of glucose, the sugar content of the plasma is approximately the same as that of whole blood. Table II, however, shows that, in the absence of cells, quite different results were obtained. In the plasma experiments the amount of bacterial growth in the diabetic and control specimens was approximately equal, or a heavier growth was obtained in the normal plasma.

#### DISCUSSION

In interpreting our results we have attempted to bear in mind that alloxan diabetes may differ in some ways from human diabetes mellitus, and that the metabolism of the rabbit differs in certain respects from human metabolism. Also that, though only some of our animals were emaciated, non-specific effects upon the defense mechanisms as result of malnutrition, such as have been described by Gellhorn and Dunn (12, 13), and Berry, Davis and Spies (14), may have had some influence. All our experiments were made with pneumococci, and the phagocytes have been found to be essential for the defense of the body against pneumococci. Rich and McKee (21) have shown that animals deprived of leucocytes will succumb to infection with even completely avirulent pneumococci. The same investigators (22) have also shown that, even in the highly immunized body, removal of the phagocytes while leaving the antibody intact destroys the body's resistance to virulent pneumococci. Wood and his colleagues (15, 16, 17, 18) have shown that while, as is well known, phagocytosis of encapsulated pneumococci does not usually take place outside the body in the absence of opsonin, *in vivo* it can occur in the complete absence of antibody if the pneumococci can be cornered in the alveoli by the leucocytes. The great importance of leucocytic action in the defense against pneumococci is thus obvious. In earlier experiments (1) we could demonstrate no defect in antibody production in alloxan diabetes, and in the experi-

ments which we have just described we were fortunate in having a pneumococcus with a small capsule, which was susceptible to phagocytosis *in vitro* in the absence of antibody. The difference between the growth of this organism in diabetic whole blood and in diabetic plasma points to some alteration in the activity of the blood cells in alloxan diabetes. It is unlikely that an alteration in the red cells is responsible for the effect, and we think that some alteration in the function of the white cells must be responsible for these results. The smears which we made during incubation did not demonstrate any complete, or even marked, loss of phagocytic power in the diabetic leucocytes, thus it seems that the defect may be an inability to destroy ingested bacteria rather than a loss of phagocytic power. At present we are quite uncertain whether this abnormality of leucocytic function is associated with excess of glycogen in the blood leucocytes, such as has been reported by Neukirch (19) in human diabetes mellitus, for we had great difficulty in staining glycogen in blood smears, and in sections of the buffy coat, stained by the Bauer method using periodic acid and leucofuchsin (20), we found no convincing difference between the normal and the diabetic leucocytes. Further studies are to be undertaken to investigate the leucocytic abnormality. The *in vitro* metabolism of the diabetic's leucocytes will obviously be a matter of particular interest.

#### SUMMARY

An encapsulated type II pneumococcus, of low virulence, when inoculated into the whole blood of rabbits with alloxan diabetes and incubated *in vitro*, grew much more rapidly than it did when incubated under similar conditions in normal rabbit blood. When these experiments were repeated using plasma instead of whole blood, growth tended to be heavier in the normal plasma. The lowered bactericidal power of the whole blood of the diabetic animals did not appear to be due to a decrease in phagocytic activity. These results are interpreted as indicating a defect in the leucocytic defense of animals with alloxan diabetes. The precise nature of the defect requires further study.

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# THE EFFECT OF EXERCISE ON THE RENAL MECHANISM OF ELECTROLYTE EXCRETION IN NORMAL SUBJECTS<sup>1</sup>

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## INTRODUCTION

The plight of the edematous patient with cardiac disease has for many years stimulated investigations into the underlying physiological mechanisms involved in the genesis of congestive cardiac failure. The plan of the study which we have undertaken is to follow the renal circulation and simultaneous electrolyte excretion in cardiac patients under varying conditions of rest and exercise. It is obvious that before conclusions can be drawn about cardiac patients, normal controls must first be studied. It is the purpose of this paper, therefore, to introduce our subject by presenting data obtained on normal controls.

For the past 30 years the backward pressure hypothesis of Starling (1) has dominated both the clinical and the research approach to the problem of congestive cardiac failure. In recent years, however, it has become apparent that more subtle factors must also play a part. The observation that the edematous state is poorly correlated with the intravenous hydrostatic pressure has led to a reconsideration of the problem.

Although Starling (1a) himself had commented on the impaired renal function in cardiac failure, it was not until 1942 that Schroeder and Fitcher (2) focused the attention of modern investigators on the

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kidneys by showing that the cardiac patient excretes less administered salt than a normal person. Two years later Warren and Stead (3) brought forth the hypothesis that lowering of the renal blood flow and diminution of the glomerular filtration rate were the primary factors in the diminished excretion of salt seen in cardiac patients.

In 1946 Landis, Brown, Fateaux and Wise (4) in animal experiments called attention to the fact that muscular exercise may play an important role in increasing the congestive state in the presence of a damaged heart.

Although every clinician is well aware of the fact that the cardiac patient is more apt to go into congestive failure while he is at home living under the varying activities and stresses of his daily life, it is surprising to find how little experimental work has been done elucidating the role of exercise in heart failure.

At the present time it seems clear that 1) the cardiac patient under certain conditions retains NaCl due to diminished renal excretion of sodium and chloride ions, 2) diminution of the renal blood flow and glomerular filtration rate does occur in cardiac failure, 3) muscular exercise increases the congestive state and worsens the condition of the cardiac patient. How these factors are interrelated and what is the exact renal mechanism in the genesis of congestive failure is the subject of the study which we have undertaken.

This report deals with thirteen experiments performed on three normal adult males, showing the changes which are induced both in the filtration rate and the excretion of plasma electrolytes by graded muscular exercise. Our interest has concerned the effect of mild exercise comparable to the average activity of sedentary workers and which the ambulant cardiac patient is capable of performing. The experiments described in this paper deal with no greater effort than walking at 3 mph on a horizontal surface. The effects of change in posture and water diuresis occurring incidentally during the experiments were recorded.

#### METHODS

The subjects were three healthy adult males, ages 28, 30 and 33. The experiments were performed in the morning, when the subjects had eaten nothing after the evening meal of the night before. No

attempt was made to regulate the salt intake in the days prior to the experiments. It happened that one of the subjects, E V N, suffered a three day attack of diarrhea due to food poisoning on one occasion, and experiments were performed in this instance on the third day of the disease and on the second recovery day. Water was allowed ad libitum up to the time of the experiment. During most experiments the subjects drank 200 ml of water after each urine collection. In several experiments all urine was collected from bedtime the night before until the end of the experiment the next morning.

The usual procedure was to have the subject void upon arrival at the laboratory in the morning. He then lay down and remained in the supine position during the control periods rising only to void. Urine collection periods varied from 10 to 30 minutes.

After two or three control periods in the supine position the test procedure was carried out. Three such procedures were used. In one, a water diuresis was induced by the ingestion of varying amounts of water, the subject remaining supine. In another, the effects of posture were studied by having the subject arise and stand quietly but not motionless for two periods. In one experiment the sitting position was tested during one collection period. Third, the effects of exercise were studied by having the subject walk on a horizontal motor-driven treadmill at the rate of 3 mph. The exercise tests were done in the winter months with the room adequately ventilated so that sweating was not perceptible. In one experiment (No 12) performed during an attack of diarrhea, the subject walked an estimated 200 yards at about 2 mph and examined a patient.

Venous blood samples were drawn during the control period, immediately following exercise, and at the end of the experiment. Heparin was used as the anticoagulant. In calculating the overnight clearances the blood levels obtained the following morning were used.

In those experiments in which inulin was used to measure filtration rate, 100 cc of an isotonic 10% solution of inulin were given in a single intravenous injection over a period of 10 to 15 minutes. Twenty to thirty minutes were allowed before the first collection period was begun. Blood was drawn before the inulin injection and at the mid-point of each urine collection period.

In the treadmill experiments, in order to measure the amount of work done, the subject's expired air was collected in a Tissot gasometer analyzed for  $\text{CO}_2$  and  $\text{O}_2$  content by Haldane's method, and oxygen consumption was calculated. Lactic acid determinations were run on blood samples drawn before and after exercise by the method of Edwards (5).

Simultaneous determinations of filtration rate (FR), and plasma clearances of sodium ( $C_{\text{Na}}$ ), potassium ( $C_{\text{K}}$ ), chloride ( $C_{\text{Cl}}$ ), and phosphate ( $C_{\text{PO}_4}$ ) were carried out on all urine specimens.

The FR was determined by the inulin clearance ( $C_{\text{In}}$ ) method of Smith (6) in each of the subjects, and this was compared with the simultaneous endogenous creatinine clearance ( $C_{\text{Cr}}$ ). Creatinine in plasma and urine was determined by the alkaline picrate method of Peters (7).

Sodium and potassium were determined on the American Cyanamide Company external standard flame photometer through the courtesy of Dr. John Eager Howard\*. Chlorides were determined by the method of Keys (8) and phosphate by the method of Fiske and Subbarow (9).

In order to avoid the injection of foreign substances which might possibly modify the electrolyte excretion pattern we have used in most experiments the endogenous creatinine clearance as the measure of glomerular filtration rate. Experiment No. 8 indicated that the injection of inulin which contains a total of 14.4 mEq of sodium could significantly modify the pattern of sodium excretion. Following the injection of inulin the  $C_{\text{Na}}/C_{\text{In}}$  rose from control levels of 0.4% to 0.6%. The ratio  $C_{\text{Cr}}/C_{\text{In}}$  obtained by simultaneous determination of plasma clearances of inulin and endogenous creatinine was constant in each of the three subjects. Values obtained for filtration rate have not been corrected to 1.73 square meters surface area.

#### METHOD OF EXPRESSION OF RESULTS

The charts are constructed to yield the greatest possible amount of information concerning the relationship between glomerular filtra-

\* For description of instrument see conference on metabolic aspects of convalescence, transactions of the eleventh meeting, Oct. 15-16, 1945, Josiah Macy Jr. Foundation, P9.



tion and tubular reabsorption of each substance as well as the magnitude of the change for each substance

An important measurement in determining the total body balance of a substance is the amount of that substance excreted in the urine. This quantity can be expressed in terms of glomerular and tubular function as the proportion or percentage of the substance filtered by the glomerulus which has not been reabsorbed by the tubules. By calculating the clearance ratios of the various electrolytes studied one may obtain values for such quantities.

The clearance ratio is  $\text{Clearance } X / \text{FR}$  where  $X$  is the substance under consideration, and FR is the filtration rate whether determined by endogenous creatinine clearance ( $C_{Cr}$ ) or inulin clearance ( $C_{In}$ ).

The ratio of the clearance of any substance to the filtration rate is the fraction of the substance filtered which is not reabsorbed and is, therefore, excreted. The reasoning behind this statement is as follows. The clearance of a substance is expressed as the number of cc of plasma which contains the amount of the substance which is excreted in the urine in one minute. Thus, if 140 mEq of sodium is excreted in the urine every minute and if the plasma level of sodium is 140 mEq/cc, then the clearance of sodium is 1 cc per minute.

Since we may assume that the concentration of sodium is the same in the plasma as it is in the glomerular filtrate we may think of the clearance of sodium as being the number of cc of glomerular filtrate which would contain the amount of sodium excreted in one minute. If the FR is 120 cc/min and the clearance of sodium is 1 cc/min, then the amount of sodium excreted is 1/120 of the amount of sodium filtered. This is the clearance ratio or the Clearance Sodium/FR. Expressed as a decimal this is 0.00833, or multiplying by 100 it becomes 0.833 per cent of the glomerular filtrate. Thus the percentage of filtered substance not reabsorbed is easily determined by the ratio of the clearance of the substance to the glomerular filtration rate. All ratios have been calculated in this way and expressed as per cent of glomerular filtrate which is excreted.

Table 1 gives the relationship, as determined by clearances, of glomerular and tubular function to NaCl balance.

Electrolyte clearance ratios have been plotted for sodium, potassium, chloride and phosphate with the ordinate on a logarithmic scale and

time as the abscissa. In order to avoid the confusion of crossing lines on the charts, each curve has been plotted separately on the same type of log paper, cut out and mounted individually. With this type of chart, if there is a greater rise or fall in the clearance ratio of one electrolyte in comparison with another, it indicates a selective change in the proportion of filtered electrolyte not reabsorbed by the tubules.

Urine concentration of any electrolyte is governed by the relative proportion of water and electrolyte filtered to that excreted. The

TABLE 1  
*Relationship of Clearances to Salt Balance*

$C_{In}$ OR $C_r$ FR cc / MIN	$C_{Na}$ cc / MIN	$\left[\frac{C_{Na}}{FR}\right]$ 100 % EXCRETED	$\left[1 - \frac{C_{Na}}{FR}\right]$ 100 % REABSORBED	DAILY TOTAL OUTPUT OF Na AS NaCl	REMARKS
100	5	5	99.5	5.8 gms	Balance
50	5	1.0	99	5.8 gms	Balance
100	0.25	0.25	99.75	2.9 gms	Salt Retention
50	0.25	0.5	99.5	2.9 gms	Salt Retention

Serum Na = 0.14 mEq / cc

Salt intake = 5.8 gms per day

This table is constructed assuming a normal filtration rate of 100 cc / min and a total intake of 5.8 gms of salt per day. The effect of a 50% reduction of FR is also shown. It is obvious that what appear to be slight changes in per cent reabsorbed would result in large changes in salt output over a 24 hour period and that slight changes in per cent reabsorbed can compensate for marked decreases in filtration rate (FR).

proportion of filtered water excreted is the ratio Urine Flow/ $C_{Cr}$  or Urine Flow/ $C_{In}$ . The relative changes in the concentrations of electrolytes may be observed by comparing the degree of rise or fall of the clearance ratios to the ratio  $UF/C_{Cr}$ . For example a fall in electrolyte clearance ratio greater than a fall in  $UF/C_{Cr}$  means a fall in concentration of that electrolyte in the urine. Thus, with this type of chart one can observe the direction of change in urinary concentration of any electrolyte as well as the relative change of the percentage of the electrolyte filtered which is not reabsorbed by the tubules. This method of interpretation, however, does not indicate the total amount of any given electrolyte in the urine unless it should happen that the filtration rate remain constant throughout the entire experiment.

## RESULTS AND DISCUSSION

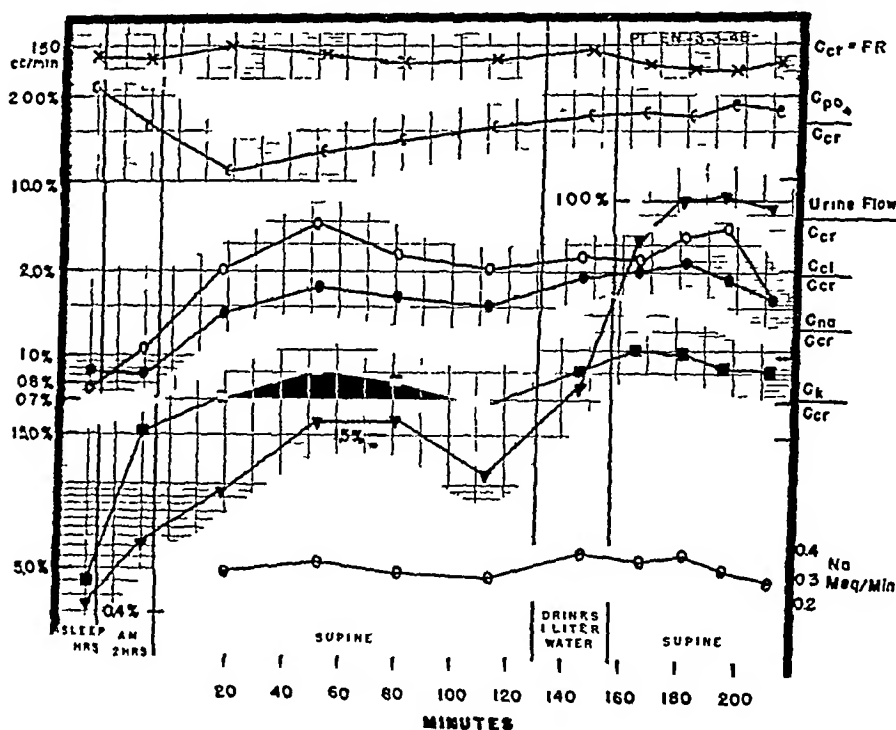
In these experiments we have attempted to correlate in the normal subject the changes in urine flow, glomerular filtration rate and electrolyte excretion which are brought about by exercise. The effects of water diuresis and posture served as controls for these observations.

TABLE 2  
*Effect of Exercise on Sodium and Water Excretion*

SUBJECT	EXPERIMENT	PRE EXERCISE		EXERCISE		HIGHEST POST EXERCISE		DURATION OF $C_{Na}/C_{Cr}$ DEPRESSION AFTER END OF EXERCISE	COMMENTS
		$C_{Na}/C_{Cr}$	UF/ $C_{Cr}$	$C_{Na}/C_{Cr}$	UF/ $C_{Cr}$	$C_{Na}/C_{Cr}$	UF/ $C_{Cr}$		
		%	%	%	%	%	%	min	
E V N	9	17	90	58	25	110	67	46	No change in FR
E V N	10	105	67	6	40	102	20	26	No change in FR
E V N	11	95	43	17	24	86	90	52	Mild G-I infection. Diuresis evident toward end of experiment. Constant FR.
E V N	12	26	74	07	50	21	64	40	Variation in FR 155-145-175 cc/min
E V N	13	144	106	48	57	130	115	23	Constant FR
S -S	6	116	110	72	73	130	94	30	Variations in FR 160-145-185 cc/min
S -S	7	55	65	11	32	46	60	36	Constant FR
S -S	8	86	92	21	37	92	100	50	Constant FR
A K	5	65	80	42	58	77	78	30	Constant FR

The most striking feature of all the experiments during mild exercise was a constant diminution in sodium excretion without a comparable change in filtration rate. Table No. 2 summarizes the essential data on the nine experiments in which the effect of exercise was tested. It is seen that  $C_{Na}/C_{Cr}$  fell consistently. The depression ranged from 17% to 65% of the pre-exercise value, average 36.4%. It was impossible to correlate the degree of depression with either the initial

control or pre-exercise value of  $C_{Na}/C_{Cr}$ . The degree of antidiuresis or the change in ratio  $UF/C_{Cr}$  had also no constant relationship

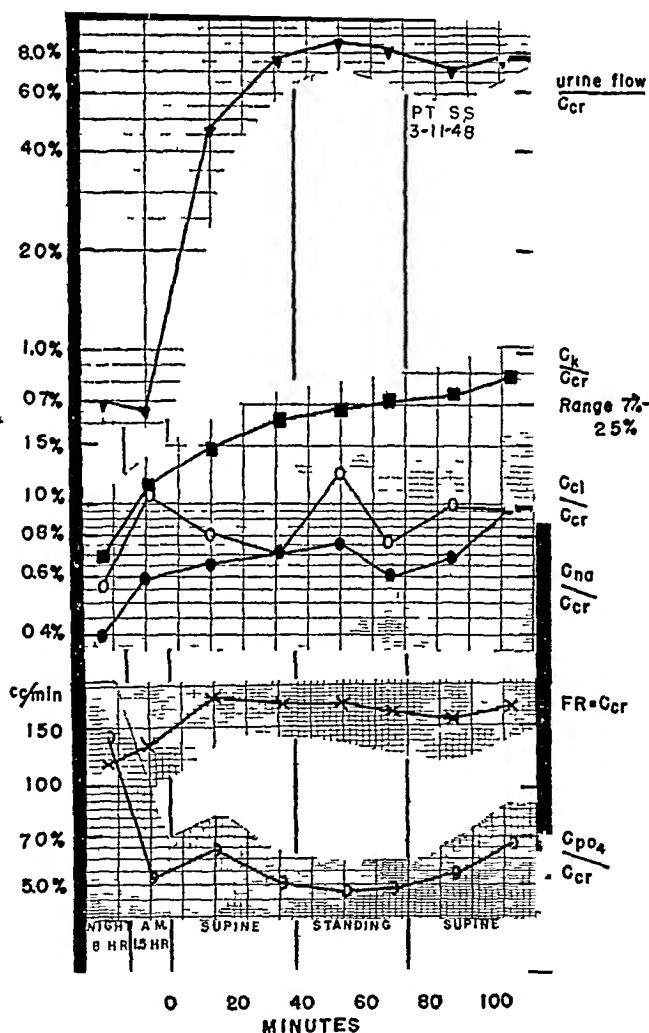


EXPERIMENT No 1

This chart shows the effect of ingestion of one liter of water on the electrolyte clearance ratios with the subject in the supine position. After rising from bed in the morning there is an increase in all clearance ratios except for  $PO_4$  accompanying a diuresis brought about by ingestion of two glasses of water, the usual amount taken by the subject during this period. In the three periods prior to the ingestion of one liter of water, electrolyte clearance ratios are constant. Despite the effect of diuresis ( $UF/C_{Cr}$  1.1% to 10%) the ratios  $C_{PO_4}/C_{Cr}$ ,  $C_{Na}/C_{Cr}$ ,  $C_{Cl}/C_{Cr}$  show only a slight rise,  $C_K/C_{Cr}$  rising from 19.5% to 30%. The filtration rate remained constant throughout the experiment.

In this case, diuresis has only a minimal effect on the excretion of sodium, chloride and phosphate.  $C_{Na}/C_{Cr}$  is virtually unaffected by a marked change in urine volume. The absolute value for the excretion of sodium in mEq/min shows no significant variation.

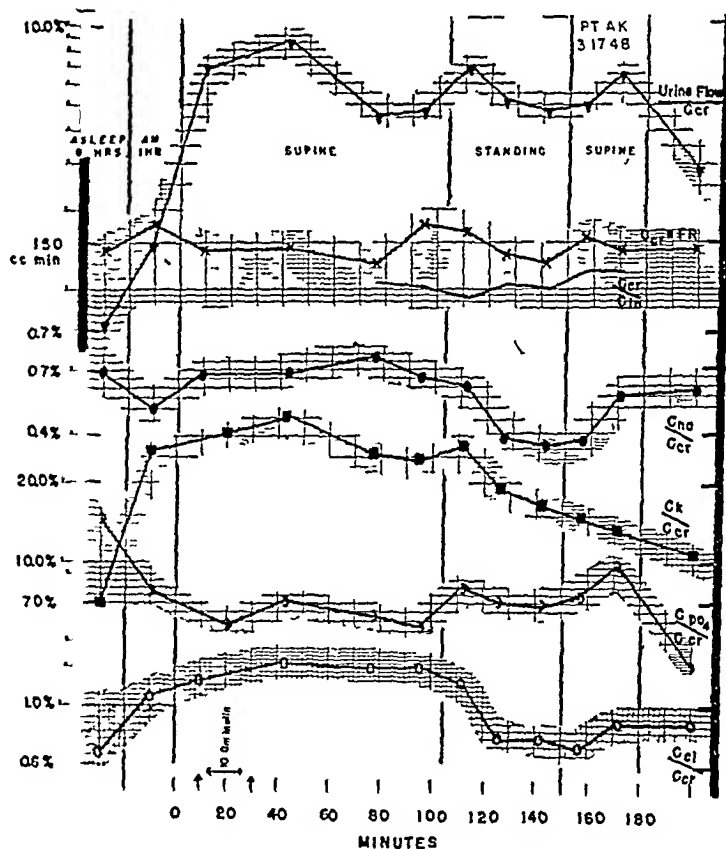
with the changes in  $C_{Na}/C_{Cr}$ . The depression of  $C_{Na}/C_{Cr}$  lasted on an average 37 minutes from the end of exercise.



EXPERIMENT No 2

This chart shows the effect of posture (standing quietly but not motionless for 35 minutes) on the filtration rate and electrolyte clearance ratios. Water diuresis was established during the supine control period in which  $UF/C_{cr}$  rose from 65% to 75%. During this time, there was a rise in filtration rate from 132 cc/min to 185 cc/min with a progressive increase in  $C_{Na}/C_{cr}$  and  $C_K/C_{cr}$ , while  $C_{Cl}/C_{cr}$  fell from 1.05% to 7%. No significant changes in FR,  $C_{Na}/C_{cr}$ ,  $C_{Cl}/C_{cr}$ , or  $UF/C_{cr}$  were noted during or after the standing period.

Excretion of chloride was inhibited in all experiments as the result of exercise, following a qualitative pattern similar to that of sodium but usually to a lesser extent. This response is opposite to that

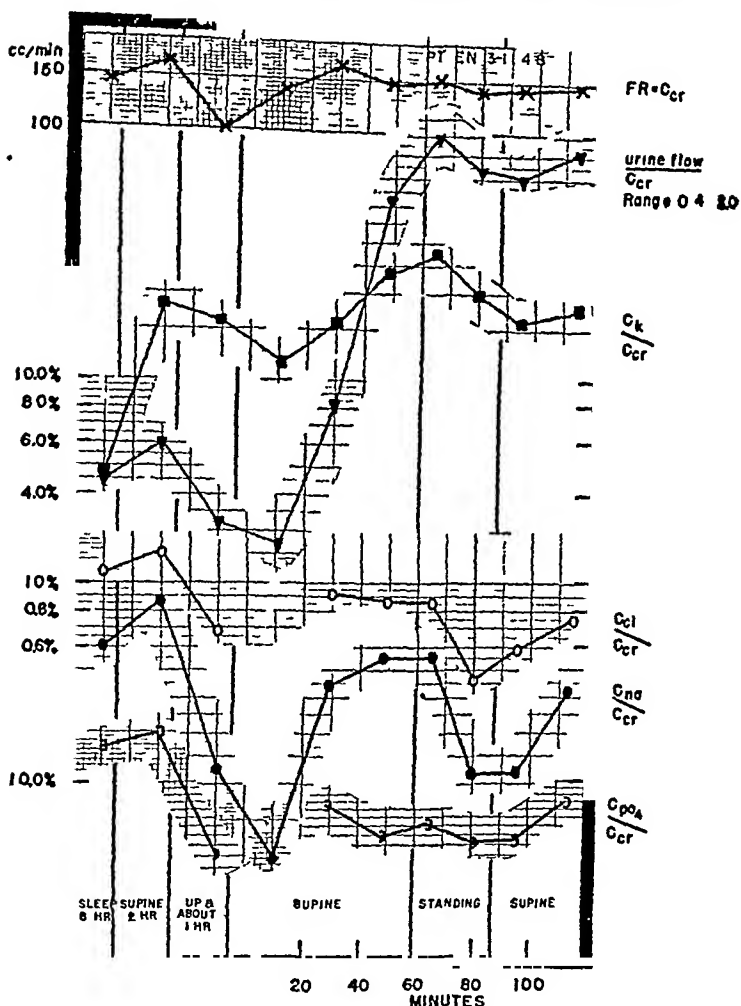


## EXPERIMENT NO 3

This chart shows the comparison of inulin and creatinine methods for the determination of filtration rate, as well as the effect of erect posture for a period of 46 minutes. Diuresis was established early in the control supine position. The increase in  $UF/C_{cr}$  from 72% to 68% was associated with a marked initial rise in  $C_{K}/C_{cr}$ , a smaller increase in  $C_{Cl}/C_{cr}$  and a depression of  $C_{Na}/C_{cr}$  from 7% to 5%. The urine flow showed marked variation throughout this experiment, the depression of  $UF/C_{cr}$  being comparable to that obtained in the control supine position. Standing in this subject produced changes of significant magnitude in  $C_{Na}/C_{cr}$ ,  $C_{Cl}/C_{cr}$ . Variations in FR are no greater than those seen during control periods. The  $C_{cr}/C_{In}$  shows little variation from unity.

In this experiment, the glomerular filtration rates determined simultaneously by inulin and endogenous creatinine clearance are of comparable magnitude, while this subject shows slight increased tubular reabsorption of both sodium and chloride while standing.  $C_{PO_4}/C_{cr}$  rose during the standing period while the  $C_{K}/C_{cr}$  fell progressively even during the recovery period.

The last period was taken two hours after the previous supine recovery period. During this time the subject had eaten his lunch.



EXPERIMENT No 4

This experiment was performed two days after the subject had recovered from an episode of diarrhea. Upon awakening in the morning the patient voided and remained in bed an additional two hours, following which he walked 500 yards to the hospital. During normal activity coming to the hospital there was significant depression of  $C_{Na}/C_{Cr}$  from 85% to 23% continuing to fall to 11% during the first supine period. Diuresis was established during the second and third supine periods,  $UF/C_{Cr}$  rising from 27% to 80%. This was associated with a recovery of the  $C_{Na}/C_{Cr}$  ratio to its overnight level. With a change in posture there was a mild antidiuresis, the sodium and chloride ratios, falling significantly, in the presence of an unchanged filtration rate and returning to their previous values by the end of the experiment.  $C_{PO_4}/C_{Cr}$  remained constant during standing while the  $C_K/C_{Cr}$  fell from 24% to 16%. The fourth urine collection was of insufficient volume to permit analysis for Cl and  $PO_4$ .

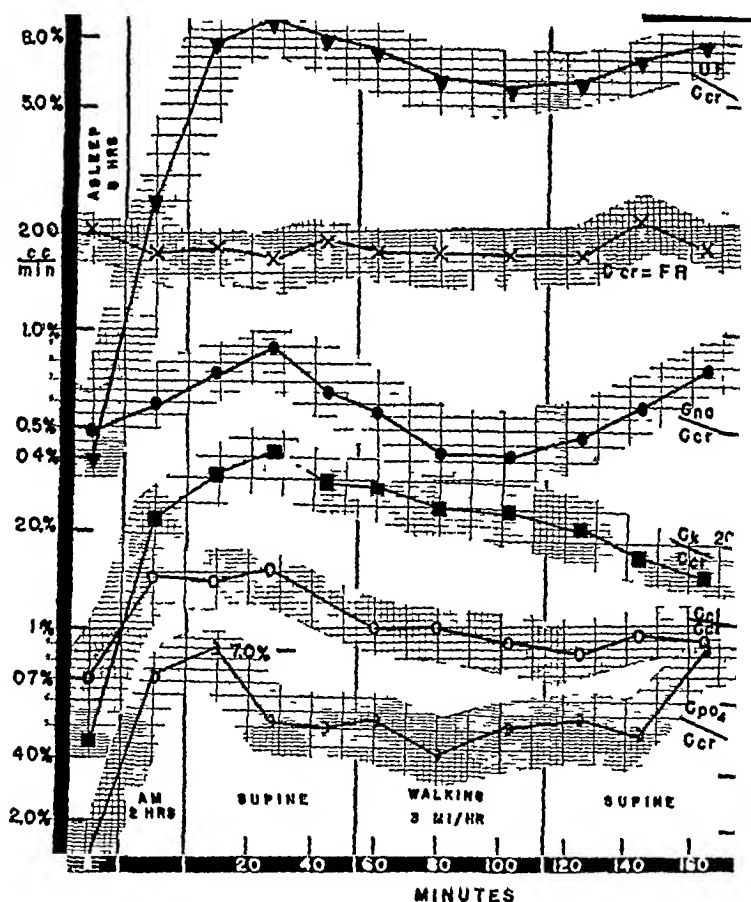
observed by Verney (10) in pituitary antidiuresis where the chloride excretion was increased. It will be noted from the charts that although both sodium and chloride excretion are reduced during mild exercise the changes in sodium excretion are of greater magnitude than those observed for chlorides. Therefore, the depression of urinary chloride cannot be taken as an absolute index of the degree of sodium retention.

The constancy of the filtration rate during mild exercise has been noted by White and Rolfe (18). Reduction in filtration rate during more severe forms of exercise has been reported by these workers and also by Barclay, Cooke, Kenney and Nutt (19), Merrill and Cargill (20) and Chapman, Henschel, Minckler and Keys (21). In all of these reports an antidiuretic effect and a reduction of urinary chloride was noted during short bursts of severe exercise. No studies of sodium excretion during severe or mild exercise have been found.

Although the depression of sodium excretion during exercise is a constant finding, certain features of this reaction should be emphasized. First, a marked variation in the response to exercise was noted in the same individual on different occasions, as is seen in Experiments No. 7 and 8. Secondly, there were marked variations in the control levels of sodium excretion in the same individual on different days. It was impossible to correlate the intensity of the response with this control level. No correlation could be found with the initial control value or pre-exercise value of urine flow. However, on the basis of two experiments (Nos. 4 and 11) performed while the subject was having diarrhea and during the recovery stage, it appears that the state of hydration and of salt depletion may modify the degree of sodium retention due to exercise. In one of these experiments (No. 4) erect posture alone was sufficient to produce a marked depression while in another (Experiment No. 11), walking produced a similar profound effect. Thirdly, the time sequence is of some interest. In several 30 minute exercise experiments the minimal level of sodium excretion was not reached until the periods immediately following the end of exercise. In all experiments an average of 37 minutes was required before the recovery to control levels was reached after cessation of exercise.

That the salt retaining response due to exercise is not a general

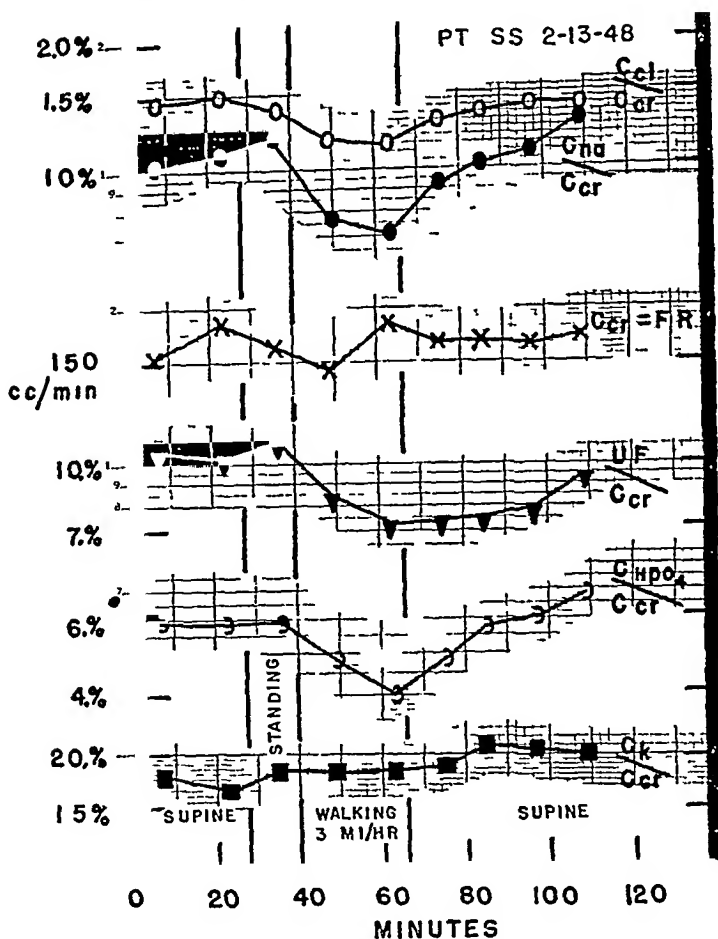




EXPERIMENT No 5

Water diuresis in this experiment was established in the control periods with the patient supine, the pre-exercise  $UF/C_{cr}$  being 8.0%. During water diuresis there was an increase in all the electrolyte excretion ratios, the  $C_{Na}/C_{cr}$  rising from 0.5% to 0.9%. Prior to walking for 60 minutes at a rate of 3 mph, the  $C_{Na}/C_{cr}$  fell to 65% and exercise produced a further depression of 42%. The filtration rate remained constant throughout the experiment. The depression of sodium excretion continued for a period of 30 minutes after the end of walking. The antidiuretic effect of exercise was not marked (8.0% to 5.8%), nor was the reduction of  $C_{Cl}/C_{cr}$ ,  $C_{K}/C_{cr}$  and  $C_{PO_4}/C_{cr}$  did not change significantly during exercise.

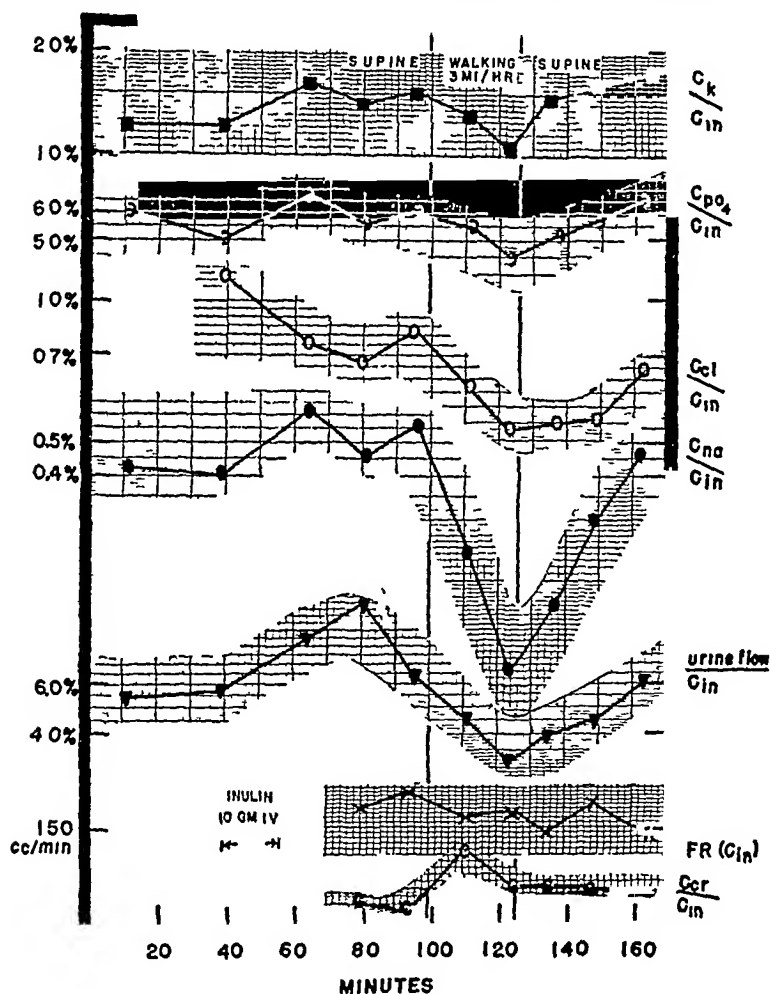
electrolyte phenomenon is shown by the clearance ratio curves of potassium and phosphate, the main intracellular ionic constituents. Although marked diurnal variation in the excretion of these elec-



EXPERIMENT No 6

This chart shows the effect of standing for 12 minutes followed by walking at 3 mph for 26 minutes. The  $UF$ ,  $Na$ ,  $Cl$ ,  $K$  and  $PO_4$  ratios were constant during the control and standing periods. The filtration rate varied from 145 to 185 cc/min throughout the experiment. During exercise,  $C_{Na}/C_{Cr}$  fell from 1.16% to .72% returning to control levels 30 minutes after the end of exercise. A persistent antidiuresis continued until the end of the experiment. The  $C_{Cl}/C_{Cr}$  showed changes similar to  $C_{Na}/C_{Cr}$  but not of the same magnitude. It will be seen that during the second exercise period when the  $FR$  was highest the sodium excretion was at its lowest level.  $C_K/C_{Cr}$  remained constant throughout the entire experiment.  $C_{PO_4}/C_{Cr}$  fell during exercise from 6.0% to 4.0%.

trolytes is apparent, no significant variations in their excretion patterns were noted which might be attributed to posture or exercise. In some experiments they rose with exercise while in others they fell

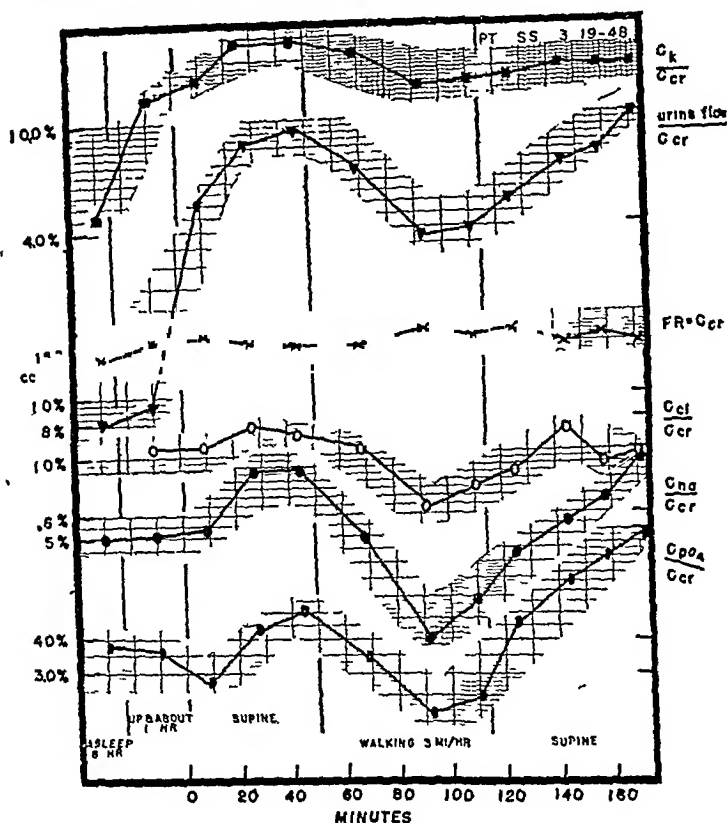


## EXPERIMENT No 7

In this experiment, filtration rate was determined simultaneously by the use of inulin and creatinine, the average  $C_{Cr}/C_{In}$  being 1.07 and the electrolyte excretion ratios are expressed relative to the clearance of inulin. Following the injection of 10 gms of inulin (containing 14.4 mEq of sodium) the  $C_{Na}/C_{In}$  increased from 0.4% to 0.6%. During exercise (walking at 3 mph for 26 minutes), the filtration rate showed no significant alteration, but  $C_{Na}/C_{In}$  fell from 0.55% to 0.11% returning only to 0.45% 30 minutes after the end of walking. Exercise produced an antidiuresis,  $UF/C_{In}$  falling from 6.5% to 3.2%, the reduction in  $C_{Cl}/C_{In}$  being of the same magnitude as the change in  $UF/C_{In}$ . Potassium and phosphate ratios showed only minor variations.

In some they remained constant. Sometimes they were parallel in their movement while at other times they moved in opposite directions.

It may be seen from the charts that the NaCl retaining response due to exercise is not due to changes in urine flow. In ten experi-

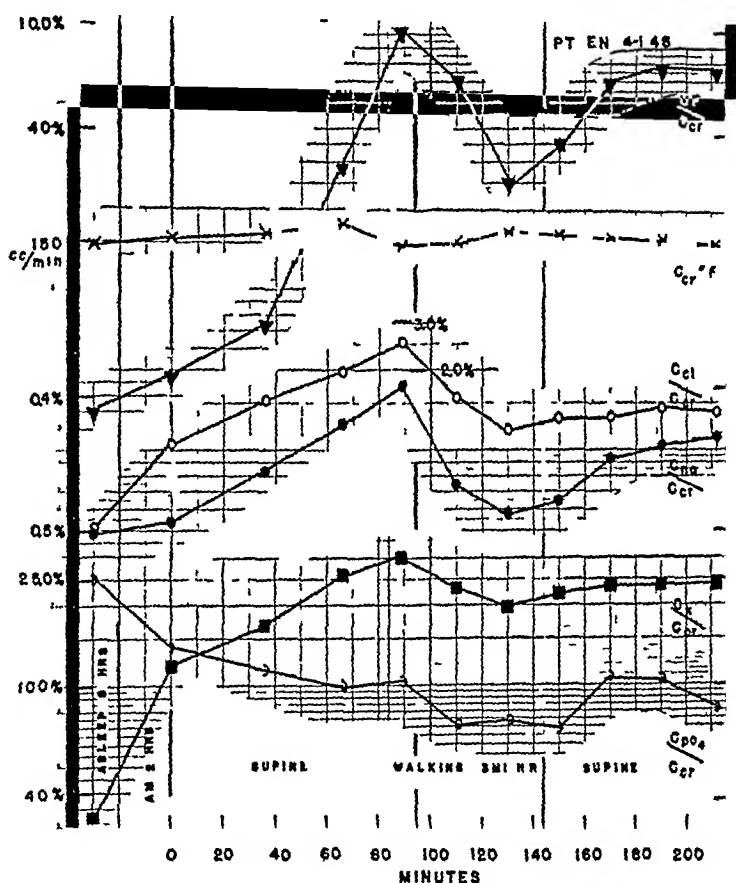


EXPERIMENT No 8

This chart shows the effect of walking for 64 minutes. Water diuresis was established during the control period, the  $UF/C_{cr}$  ratio rising from an initial value of 0.8% to 9.2% prior to exercise. Associated with this diuresis, there was a marked rise in  $C_K/C_{cr}$ , with a less marked increase in  $C_{Na}/C_{cr}$  and  $C_{Cl}/C_{cr}$ . The filtration rate remained constant throughout the experiment. Exercise caused an antidiuresis (9.2% to 3.7%) while  $C_{Na}/C_{cr}$  fell from a control level of 0.86% to 0.21%, rising only to its former level 50 minutes after the end of walking.  $C_{Cl}/C_{cr}$  showed the same trend, although the degree of depression was not so great. While  $C_K/C_{cr}$  was very little affected by exercise,  $C_{PO_4}/C_{cr}$  fell during exercise to about 50% of the pre-exercise level, a change of the same magnitude as  $UF/C_{cr}$ .

ments a water diuresis was observed. In no case was any significant depression of sodium or chloride excretion observed coincident with

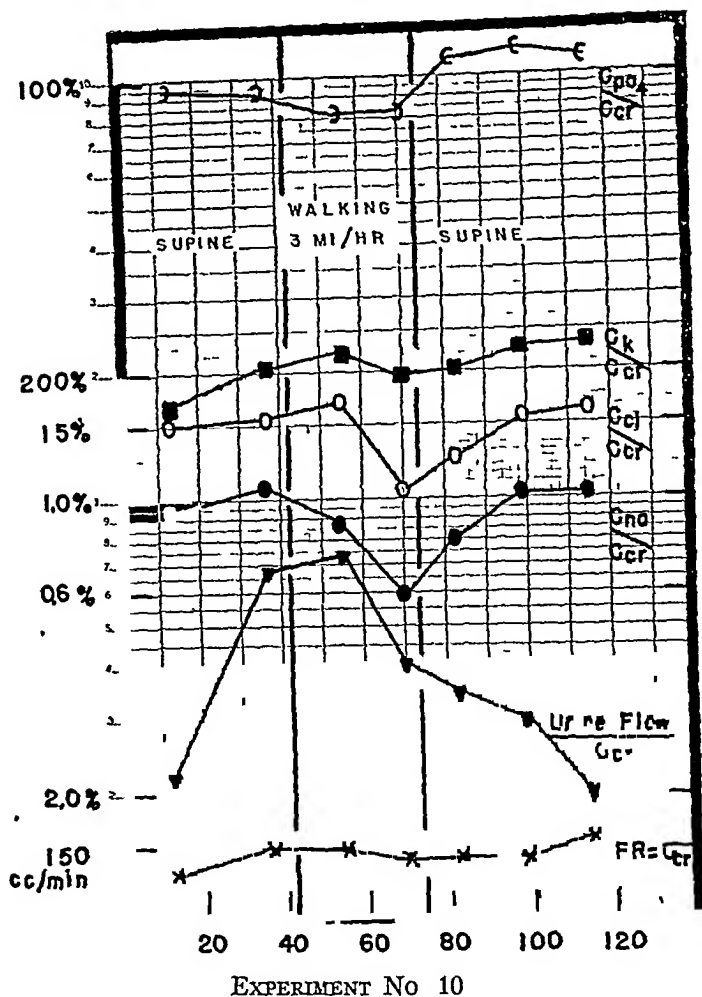
water diuresis induced by the ingestion of an excess of water. In Experiments Nos 1, 2, 3, 5 and 9 the establishment of diuresis in the



EXPERIMENT No 9

This chart shows the effect of walking at 3 mph for 60 minutes, water diuresis having been established at a  $UF/C_{cr}$  ratio of 9.0% at the beginning of exercise. This diuresis was associated with a constant filtration rate but with an increase of the electrolyte clearance ratios,  $C_{K}/C_{cr}$ ,  $C_{Na}/C_{cr}$ ,  $C_{Cl}/C_{cr}$  and a decrease in the  $C_{PO_4}/C_{cr}$  ratio. Exercise produced no change in filtration rate, but a progressive depression of  $C_{Na}/C_{cr}$  from 1.7% to 0.58%, the ratio returning to 1.05% 46 minutes after the patient had finished walking. This degree of exercise produced a definite antidiuresis, and at the same time, the  $C_{Cl}/C_{cr}$  mirrored the sodium effect, the change being of small magnitude. Potassium and phosphate ratios were slightly depressed during exercise.

morning was accompanied by an increase in the sodium and chloride excretion over the overnight controls. In Experiment No 1 a large water diuresis after the morning urine flow had been established



EXPERIMENT No 10

Diuresis was established in the initial control periods, with the subject supine,  $UF/C_{cr}$  rising from 2.2% to 6.7%. The electrolyte ratios  $C_K/C_{cr}$ ,  $C_{Cl}/C_{cr}$ ,  $C_{Na}/C_{cr}$  remained constant. During 32 minutes of walking at 3 mph, the filtration rate remained constant, while an antidiuresis caused a fall of  $UF/C_{cr}$  to 4.0%. The  $C_{Na}/C_{cr}$  fell from 1.1 to 0.6% returning to its control level 26 minutes after the end of exercise.  $C_K/C_{cr}$  showed little alteration. The antidiuresis continued until the end of the experiment, despite the constancy of the filtration rate, while the chloride  $C_{Cl}/C_{cr}$  followed the sodium curve. No water was drunk after the pre-exercise control periods, showing in this instance that the depression in sodium excretion returned to control levels with a falling urine flow. The potassium and phosphate ratios showed only minor variations.

caused no significant change in the rate of sodium or chloride excretion. In Experiment No 10 it is seen that antidiuresis itself does not cause the NaCl retaining response because a decline in urine flow due to a

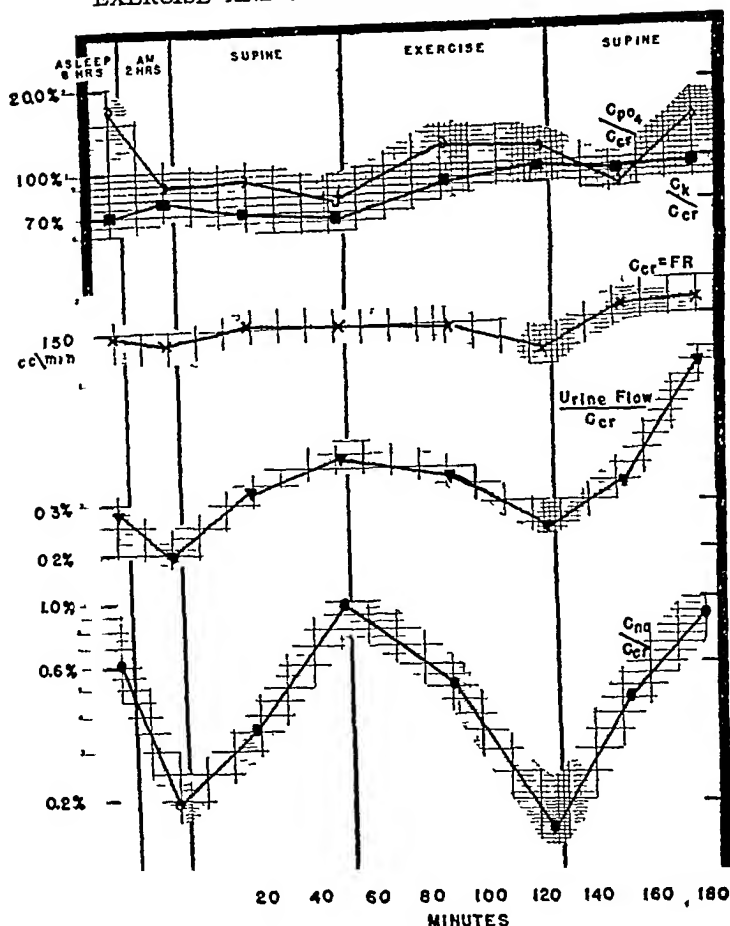
restriction of water intake occurred while the sodium clearance ratio was increasing coincident with recovery from the exercise period

The glomerular filtration rate remained constant during water diuresis. Other investigators studying the effect of diuresis or electrolyte excretion have obtained somewhat variable results. Marshall (11) showed that during a water diuresis no measurable increase in creatinine excretion occurred but total urinary chlorides were increased. Priestley (12) noted a transitory rise followed by a progressive fall in urinary chlorides. Eggleton (13) found a diminution in chloride excretion while Barclay and Nutt (14) found marked variation, stating that the degree of hydration of the individual subject at the time of diuresis conditioned the extent of resultant chloride excretion. Crutchfield and Wood (15) in a study of water diuresis (Schemm regime) as a form of therapy for congestive cardiac failure suggest that in non-cardiac patients the sodium excretion following the ingestion of large quantities of water is a function of the initial control level of the urine flow (16).

Our results indicate that large variations in urine flow do not appreciably modify the pattern of electrolyte excretion during the short duration of these experiments.

Since the exercise experiments necessitated changing from the supine to the erect posture it became necessary to study also the changes in renal hemodynamics, urine flow and electrolyte excretion which might be brought about by changes in posture. Such changes have not been extensively investigated. Braun, Knudsen and Raschau (17) studied changes in renal hemodynamics during passive erect posture on a tilt table. They noted a fall in the renal blood flow and filtration rate as well as an antidiuresis in normal subjects when changing from the horizontal to passive erect posture. These subjects, however, were suspended by their perineums in a saddle-like arrangement so that there was no muscular activity in the legs to preserve an adequate venous return from the lower extremities. White and Rolfe (18) observed a diminution of 8% in the filtration rate on normal subjects when changing from supine to erect posture.

We have observed no alterations either in electrolyte excretion or in renal hemodynamics when changing from the supine to the sitting posture during a 20 minute period of time (Experiment No. 13).



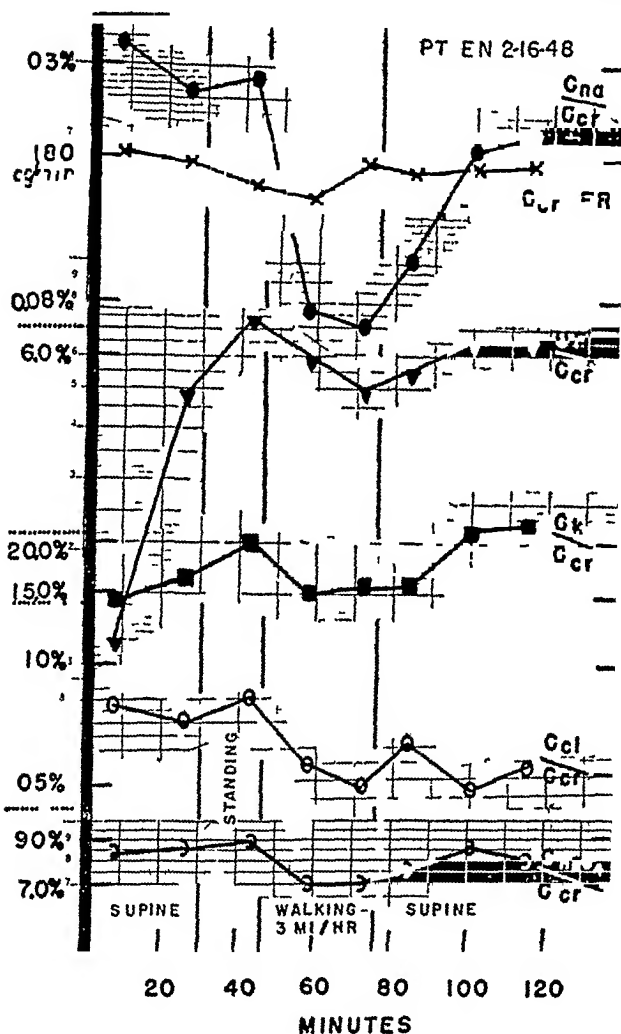
## EXPERIMENT No 11

This experiment was performed while the subject was suffering from an attack of diarrhea, the urine flow and sodium clearance being at a low level. Coming to the hospital (A M 2 hours) is associated with a fall of  $C_{Na}/C_{Cr}$  from 0.6% to 0.2% which returns to its overnight value during the control supine period. Exercise (walking to a consultation and back to the laboratory) caused some antidiuresis while a depression of  $C_{Na}/C_{Cr}$  is seen similar to that previously obtained. These effects return to the previous control level in the following 52 minutes.

The constancy of the filtration rate in the presence of marked variations of  $C_{Na}/C_{Cr}$  is a striking feature. The presence of a mild gastro-intestinal disturbance has, presumably, caused  $C_{Na}/C_{Cr}$  and  $UF/C_{Cr}$  to be of low value. Potassium and phosphate ratios remained constant during exercise.

However, changing from the supine to the standing position brought about a small but definite antidiuretic effect and a small depression of sodium excretion (Experiments No 2, 3, 4, 6 and 12).

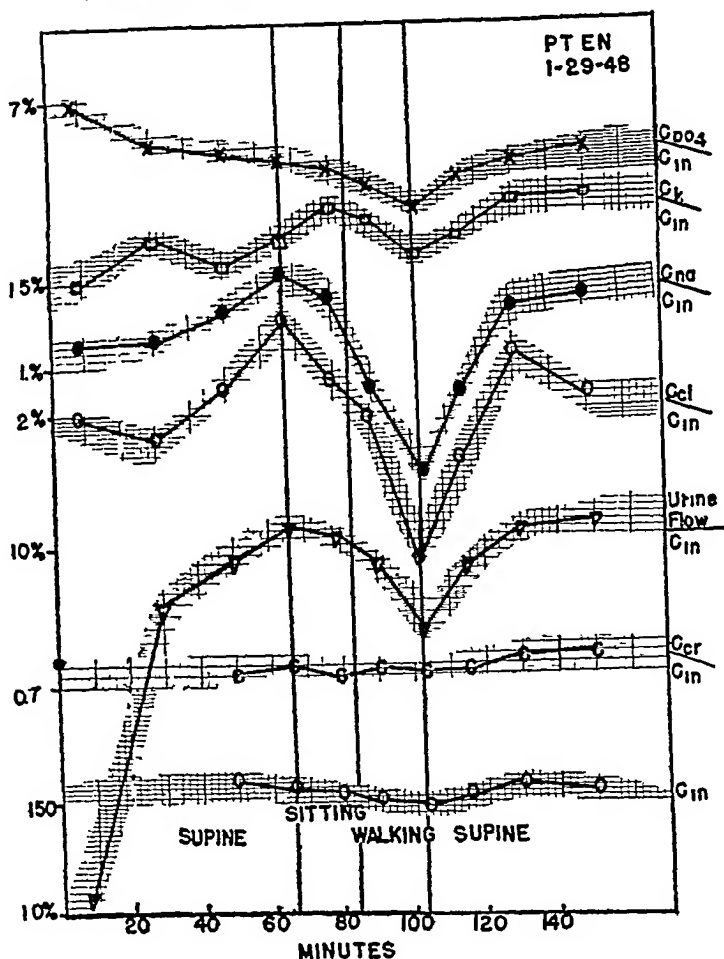




EXPERIMENT No 12

This chart shows the effect of water diuresis, posture, and walking at 3 mph for 30 minutes. During the initial 30 minutes,  $UF/C_{Cr}$  rose from 1.2% to 7.4%. The  $C_{Na}/C_{Cr}$  fell from 0.34% to 0.26% with a slight rise in  $C_{K}/C_{Cr}$ . During exercise  $C_{Na}/C_{Cr}$  fell from 0.28% to 0.07%, this being accompanied by a mild antidiuresis, 7.3% to 5.0%. The filtration rate showed little if any variation, while the ratio  $C_{Na}/C_{Cr}$  had not returned to the control level by the end of the experiment, 40 minutes after the end of exercise. Slight depression occurred in the potassium and phosphate ratios comparable in magnitude to the depression in  $UF/C_{Cr}$ .

Oxygen consumption was determined during exercise in the experiments and corrected to 1.73 Sq M of surface area. Each subject



EXPERIMENT No 13

Diuresis was established during the supine control period  $UF/C_{Cr}$  rising from 11% to 110%. During this time, the ratios of  $C_{Cl}/C_{In}$ ,  $C_K/C_{In}$  and  $C_{Na}/C_{In}$  increased to some degree, and the effects produced by a change of posture are further accentuated by walking for a period of 20 minutes at 3 mph. An anti-diuresis occurred,  $UF/C_{In}$  falling from 11% to 0.8%. The  $C_{Na}/C_{In}$  and  $C_{Cl}/C_{In}$  ratios showed a marked depression,  $C_{Na}/C_{In}$  falling from 1.7% to 0.48% nearly a four fold change. Filtration rate fell from 160 to 150 during exercise. The ratio  $C_{Cr}/C_{In}$ , although not unity, showed constancy through the experiment, ranging from 0.7 to 0.8. The potassium and phosphate ratios showed slight depression during exercise.

showed marked constancy, the range for 19 determinations being 0.875 to 1.18 with an average of 1.07 l/min / 1.73 Sq M body surface area.

Two determinations of oxygen consumption were made on each subject, one during the first half and the other during the second half of the exercise periods. The values were constant for each subject throughout the exercise period.

Blood lactic acid levels determined during and immediately after exercise showed no significant variation from the pre-exercise control level.

Serum sodium and potassium levels were taken in each experiment before and after exercise and in each instance they remained constant within the limits of error in the method.

Three possible mechanisms by which this NaCl retaining response might be mediated have been considered.

1) The possibility that there was increased reabsorption of sodium to compensate for that lost by sweating has been minimized by conducting the experiments under conditions where sweating was not perceptible.

2) Substances liberated from the tissues during exercise might affect the renal tubule. An increase in blood lactic acid is not responsible, and we assume that anaerobic muscle work is not necessary to elicit this renal tubular retention of sodium.

3) Hormonal influences either of the adrenal cortex or posterior pituitary may mediate this response. If the specific tubular retention of sodium which occurs during exercise can be regarded as an adaptation phenomenon, further experiments should be done to determine what physiological function this effect serves. At this stage we feel justified only in stating that mild exercise which produces no measurable change in glomerular filtration rate evokes a NaCl retaining response. The mechanism of this phenomenon will be the subject of further study in normal subjects. Its implication in the genesis of congestive cardiac failure will be the subject of a further report.

#### CONCLUSIONS

1) Thirteen experiments on three normal adult males have been reported to show the effect of standing and walking on the glomerular filtration rate and the renal mechanism of sodium, chloride, potassium, phosphate and water excretion.

2) The exercise was walking at 3.0 mph on a motor driven tread-

mill The average oxygen consumption during this work was 1 07 l/min /1 73 Sq M body surface area (range 0 88-1 18) There was no increase in blood lactic acid due to this work

3) This exercise produced no significant alteration in glomerular filtration rate or serum sodium and potassium concentrations

4) Exercise caused a specific sodium and chloride retention due to a decrease in the proportion of filtered sodium excreted or an increase in the tubular reabsorption of filtered sodium The retention persisted an average of 37 minutes after the end of exercise

5) Standing quietly has a slight sodium and chloride retaining effect

6) A variable antidiuresis occurred with exercise

7) The potassium and phosphate excretion did not show any predictable correlation with the changes in sodium excretion

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# THE RENAL MECHANISM OF ELECTROLYTE EXCRETION AND THE METABOLIC BALANCES OF ELECTROLYTES AND NITROGEN IN CONGESTIVE CARDIAC FAILURE, THE EFFECTS OF EXERCISE, REST AND AMINOPHYLLIN<sup>1</sup>

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## INTRODUCTION

The increase in extracellular fluid which occurs in congestive cardiac failure has emphasized the importance of sodium excretion in this condition. This paper reports observations on three cases of congestive cardiac failure and will indicate the influence which the renal tubules exert on the excretion of the main extracellular fluid cation.

The mechanism of edema has been explained on the basis of altered hydrostatic and osmotic relationships in the peripheral vessels and the appearance of edema in cardiac decompensation has been accepted as a clinical sign of myocardial failure (1) (2) (3) (4) (5) (6). The knowledge that sodium excretion is impaired in congestive failure (7) (8) (9) has suggested that the hypervolaemia of this condition may, in part, result from abnormal renal function (10). Diminished renal blood flow and glomerular filtration rate, with a high filtration fraction, has been found to occur during cardiac decompensation (11). These circulatory changes within the kidney have been accepted as causing the abnormal retention of salt and water whereby the amount of sodium conveyed to the kidney for excretion is diminished—"The decreased excretion rate of sodium in congestive cardiac failure is attributable to a decreased filtration rate in the presence of a normal tubular mechanism" (12). The observations reported in this paper

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suggest that the increased reabsorption of filtered sodium by the renal tubules may play a part in the accumulation of extracellular sodium, equally important as that due to a decrease in the absolute amount of sodium which is filtered by the glomeruli

Aminophyllin and mild muscular exercise produce opposing effects on the excretion of sodium by their action on the renal tubules (13) (14) Mild muscular exercise in normal subjects causes, in the presence of an unchanging filtration rate, a specific sodium retaining response Aminophyllin, in the absence of changes in the renal circulation, produces decreased tubular reabsorption of sodium and chloride This paper will report (1) the effects of aminophyllin and mild muscular exercise in one case of congestive cardiac failure while on metabolic balance studies and (2) the effect of mild muscular exercise on two other cases of congestive cardiac failure

#### CASE REPORTS

*Patient No 1*, L S, colored female, age 38 years, surface area 1.62 sq m

Admitted to the Medical Service of The Johns Hopkins Hospital on November 7, 1947 in her first attack of congestive cardiac failure due to rheumatic mitral stenosis and aortic insufficiency Marked peripheral edema was present Blood pressure, 146 systolic, 76 diastolic mm Hg, weight 78 kg, venous pressure 196 mm water There was a history of intermittent digitalis therapy over the preceding three months with no dietary salt restriction Observations on this patient are divided into three periods

*Period No 1* (21 days) November 7, 1947 to November 28, 1947 Experiments No 1, 2 and 3 were performed during this period Prior to Experiment No 2 the patient had been digitalized and had received two injections of 2 cc of mercuripurin All diuretics were discontinued from this time Maintenance digitalis 0.1 gm daily was commenced and continued throughout this study At the end of this period, some pitting edema of the ankles still existed and the venous pressure was 100 mm water Diet during this period contained 2 gm salt

*Period No 2* (24 days) November 28, 1947 to December 22, 1947 Patient was transferred to Metabolic Ward of the Medical Division and placed on balance studies for nitrogen, sodium, potassium and chloride Maintenance digitalis 0.1 gm daily was given, no diuretics being used From Day 1-15 diet contained 1 gm salt From Day 16-23 an additional 5 gm of salt were added to the food Experiments No 4, 5 and 6 were performed on Days No 7, 12 and 21 By the end of this period, the patient was edema-free, weight was 60.0 kg and the venous pressure was 10 mm water

*Period No 3* (92 days) December 22, 1947 to March 23, 1948 Patient followed in Out-Patient Department Therapy consisted of 0.1 gm digitalis daily, no

diuretics were used and a voluntary salt restriction of 2 gm daily was attempted. Clinical evidence of congestive failure with edema was progressive from the end of January, 1948. By February 27, 1948, weight was 73 kg, venous pressure 115 mm water and ankle edema was present. Experiments No 7, 8 and 9 were performed on Days No 67, 74 and 92.

*Patient No 2*, L H, white male, age 50 years, surface area 1.68 sq m

Patient admitted to the Medical Service of The Johns Hopkins Hospital on April 15, 1948 in his first attack of congestive cardiac failure which had developed following an anterior myocardial infarction six weeks previously. The patient had been digitalized two weeks before admission and 0.1 gm of digitalis daily was continued while in the hospital. No diuretic drugs were given. On admission weight was 63 kg, peripheral venous pressure 87 mm water, blood pressure 118 systolic, 74 diastolic mm Hg. Experiment No 10 was performed on April 20, 1948 at which time the patient was dyspneic on exertion, paroxysmal nocturnal dyspnea was present, and 1+ ankle edema existed.

*Patient No 3*, B M, white male, age 30 years, surface area 1.91 sq m

Patient admitted to the Medical Service of The Johns Hopkins Hospital December 11, 1948 with a history of recurrent attacks of exertional dyspnea, orthopnea, abdominal swelling and ankle edema of one and a half years duration. Patient had suffered from rheumatic fever at the age of 11 and 18 years. Main physical findings at the time of admission included auricular fibrillation (apical rate 72/min with pulse deficit 23/min) blood pressure 110 systolic mm Hg, cardiac enlargement with apical impulse in the fifth left intercostal space 11 cm from the midsternal line with a mid diastolic murmur heard within the area of the apex beat. The liver was enlarged 8 cm below the right costal margin and minimal ankle edema was present. At the time of admission the patient was receiving digitoxin 0.1 mgm daily and no diuretics had been used for a preceding two weeks. Experiment No 11 was performed on December 15, 1948, at which time the patient was markedly dyspneic on exertion.

#### METHODS

The methods used in these studies have been previously described (15). Filtration rate was determined either by the inulin or endogenous creatinine clearance methods. Renal plasma flow was measured by the clearance of para-acetylaminohippuric acid (PACA) on a falling plasma concentration curve after a single intravenous injection. Electrolyte excretion has been expressed either by clearance or clearance ratios. Clearance ratios are used in order concisely to represent the changes in urinary electrolytes in terms of the relationship between the amount filtered through the glomeruli and the amount reabsorbed by the tubules. The expression 'clearance ratio' used in



this paper means the percentage of filtered electrolyte not reabsorbed by the renal tubules This expression is  $\frac{C_{(\text{electrolyte})}}{C_{(\text{inulin})} \text{ or } C_{(\text{creatinine})}} \times 100$

If a fall in filtration rate below normal occurs with a constant clearance ratio, the decrease in quantity of electrolyte in the urine will be proportional to the decrease in filtration rate Decreases in electro-

TABLE 1

*Patient L S Renal Hemodynamic Changes and Blood Chemical Findings*

DAY	EX PERI MENT	FILTRA- TION RATE	RENAL PLASMA FLOW	FILTRA- TION FRAC TION	PLASMA Na mEq/l	PLASMA K mEq/l	PLASMA Cl mEq/l	PLASMA PROTEINS			NPN	SERUM PHOS PHO RUS	VE NOUS* PRES SURE mm H <sub>2</sub> O
								Tot	Alb	Glob			
Period No 1 (11/7/47-11/28/47) 21 Day Period of Cardiac Compensation													
		cc/min	cc/min					gm %	gm %	gm %	mg %	mg %	
1	1	101	187.5	54	138	4.2	115	6.4	3.1	3.3	28	4.8	196
5	2	106	269	39	135	4.0	107	7.5	3.8	3.7	26		130
14	3	117	267	44	136	4.6	113	7.3	4.0	3.3	30	4.3	100
Period No 2 (11/28/47-12/22/47) 24 Day Period of Metabolic Balance Study													
1					138	4.2	104	7.6	3.9	3.7	36		
4		85	267	40	140	5.1	115.8				26		70
7	4	108	347	31			110						50
12	5	94	302	31	139	4.3	106	7.3	4.0	3.3	30		25
21	6	99.2	417	23	148	4.5	115	7.9	3.8	4.1	31		10
Period No 3 (12/22/47-3/23/48) 92 Day Period of Cardiac Decompensation													
67	7	127	255	49	144	3.4	115					4.3	115
74	8	94			131	4.0	112					4.1	185
92	9	100			135	4.2	103	7.1	3.9	3.2	29	5.1	118

\* All venous pressure for this patient were measured from a constant arbitrary zero point, the patient being elevated at 40° to the horizontal

lyte output are then due primarily to a fall in filtration rate without a change in the relationship of renal tubular reabsorption to filtration rate If there is an increase in the tubular reabsorption of electrolyte above the proportion previously or normally found, the ratio will fall, whether there has been a fall in filtration rate or not

#### *Renal Function during Cardiac Compensation*

Table 1 shows relevant data for the Periods No 1, 2 and 3 throughout the study of Patient No 1, L S

*Period No 1* Glomerular filtration rate was not significantly depressed during the acute stage of decompensation (normal range for this patient  $109 \pm 14.6$  cc/min), although renal plasma flow was low with a filtration fraction of 0.54. During the period of digitalis administration, there was a progressive rise in renal plasma flow with a resultant fall in the filtration fraction. The sodium clearance when determined on the first hospital day at bed rest was 1.02 cc/min. Plasma sodium levels varied between 136–138 mEq Na/l. Peripheral venous pressure fell from 196 to 100 mm water.

*Period No 2* shows similar changes occurring during the period in which the patient was followed on metabolic balance studies. On Day 21 the renal plasma flow was 417 cc/min with a filtration fraction of 0.23. Plasma sodium levels were within normal range (138–148 mEq Na/l). Following the addition of an extra 5 gm of sodium chloride to the diet on Day No 16, the patient at this stage of cardiac compensation was able to excrete all of the extra amount of sodium.

*Period No 3* presents data during the out-patient observation of this patient when her weight had risen from 60 to 73 kg and her venous pressure from 10 to 118 mm water. Filtration rate varied between 94–127 cc/min but in one determination made on renal plasma flow, the filtration fraction had risen to 0.49. Plasma sodium concentration (131–135 mEq Na/l) were at the lower limits of normal. Sodium clearances diminished from 1.0 to 0.23 cc/min.

#### *Metabolic Disturbances Observed during Cardiac Failure*

The results obtained during Period No 2 (Patient L. S.) are represented in chart No 1 by the method advocated by Albright (16). Significant findings are

- 1 The initial positive nitrogen balance persisted until the 19th day and averaged 3 gm nitrogen/day.

- 2 Plasma albumin and globulin concentrations were within normal range.

- 3 Positive potassium balance occurred during the period of positive nitrogen balance.

- 4 Negative sodium and chloride balance existed until the end of the 15th day and was at that time, of the order of 40 mEq/day. On the 16th day an additional 5 gm of salt were added to the diet and positive sodium and chloride balance occurred on the 16th and 17th

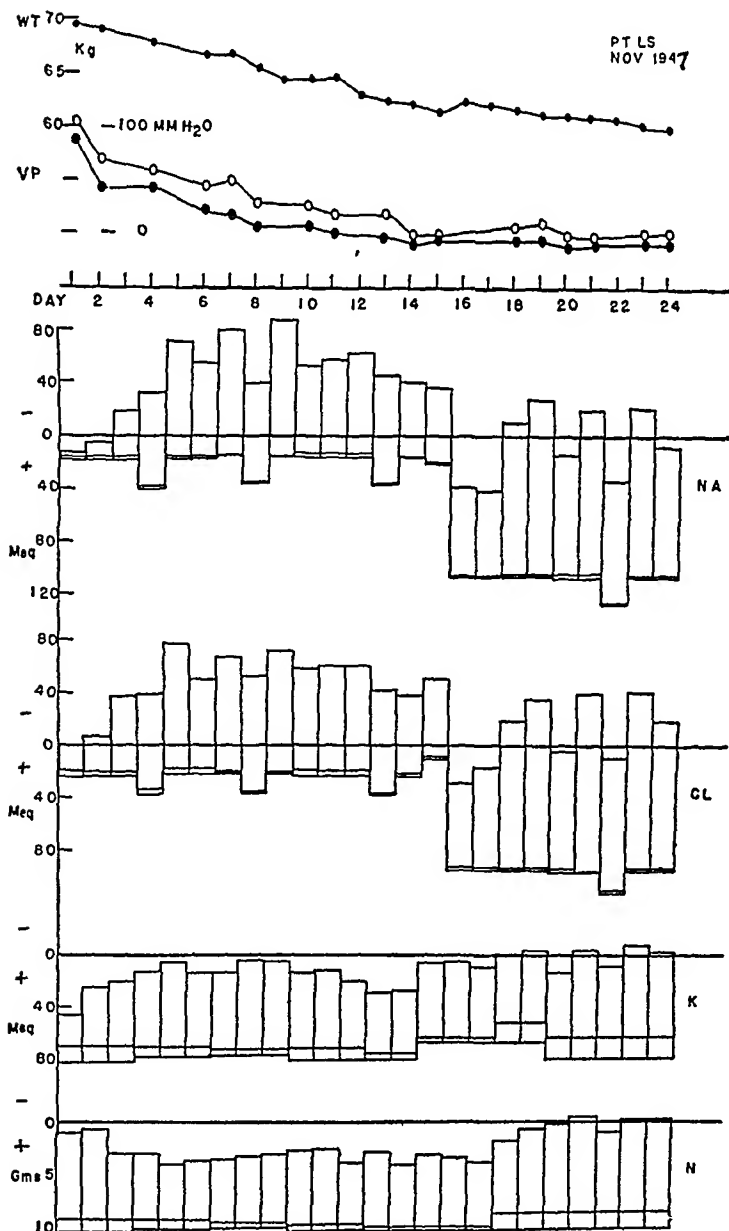


CHART No 1

METABOLIC BALANCE DATA OBTAINED FROM PATIENT WITH  
CONGESTIVE CARDIAC FAILURE

*Patient No 1 L S (Period No II)*

This chart shows changes in weight, venous pressure and balance data for sodium, chloride, potassium and nitrogen during Period No II. The scale for

day Thereafter until the end of the experimental period, sodium and chloride equilibrium existed, the extra salt being all excreted Filtration rate was 99.2 cc/min, and the sodium clearance was 1.07 cc/min At this point, the patient was almost edema-free

5 Weight decreased progressively from 69.5 kg to 60.0 kg

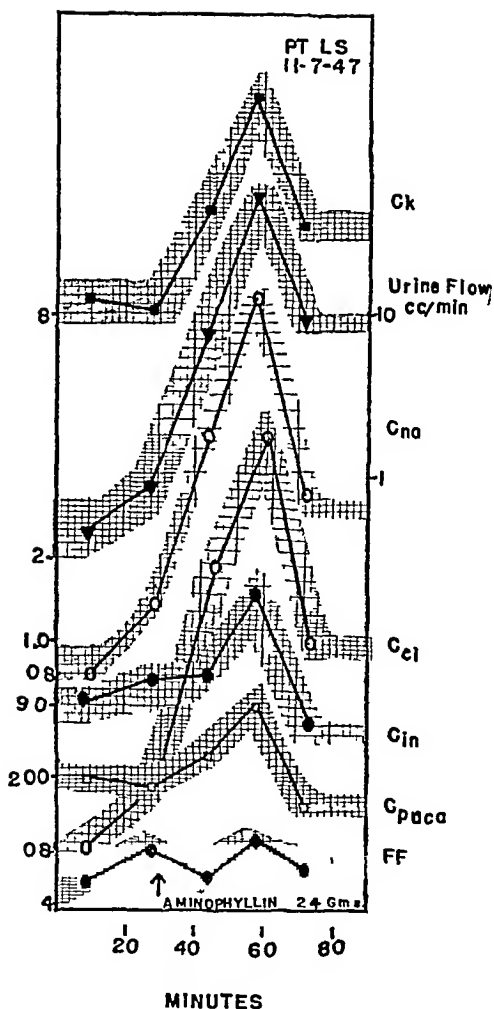
Although hypoproteinemia was not present in this case, positive nitrogen balance was present from the first to the 19th day of the observation period During the period of recompensation, negative sodium and chloride balance existed, the positive potassium balance closely following that of nitrogen Assuming that 2.7 mEq of potassium is associated with 1 gm of nitrogen in protoplasm (16) (17) and deriving the amount of potassium added to intracellular fluid in protoplasm from the nitrogen balance, all of the retained potassium could not be accounted for on the basis of protoplasm synthesis It would be interesting to know whether changes in the base-binding power of protoplasm undergo alteration in cases of congestive cardiac failure with sodium in part replacing potassium as the main intracellular cation

#### *Action of Aminophyllin in Congestive Cardiac Failure*

Experiments No 1-3 demonstrate the dual action of aminophyllin as a diuretic in congestive cardiac failure In Experiment No 1 when the patient was in marked failure and not completely digitalized, aminophyllin 0.24 gm administered intravenously caused lowering of the venous pressure, increased renal blood flow and glomerular filtration rate, probably secondary to increased cardiac output How-

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intake and balance in gm or mEq/24 hr is given as the ordinate, time is expressed in days as the abscissa The horizontal line at 0 of the ordinate is the base line to which intake and balance refer Intake is plotted as an area from the base line toward the bottom of the diagram, excretion is represented by a horizontal line measured from the bottom of the intake line The upper line for venous pressure is in the supine position, the lower for a standard 40° vertical position During this period weight fell from 69.5 kg to 60.0 kg, venous pressure (supine) from 100 to 10 mm water From Day 1-15 negative sodium and chloride balance existed From the 15th day an extra 5 gm of salt was added to the diet This was all excreted by the patient in the subsequent days From Day 1-19 positive nitrogen and potassium balance occurred concurrently, the positive nitrogen balance being approximately 3 gm nitrogen per day



EXPERIMENT No 1

*Patient No 1 L S*

In this experiment, filtration rate, renal plasma flow and simultaneous clearances of sodium, chloride and potassium are shown for two periods before and three after the intravenous administration of 0.24 gm aminophyllin. In the first collection period following the injection, there is approximately a three-fold increase in sodium, chloride and water excretion with little change in filtration rate or renal plasma flow. In the next collection period, there is a two-fold rise in renal plasma flow and filtration rate. At the same time, the sodium and water excretion is increased seven-fold and the chloride excretion ten-fold above the pre-injection control level. In the last period the renal blood flow and glomerular filtration rate have fallen to their control values, but the sodium and chloride excretion and urine flow still remain elevated. Following the injection of aminophyllin, the venous pressure fell from 196 to 150 mm water.

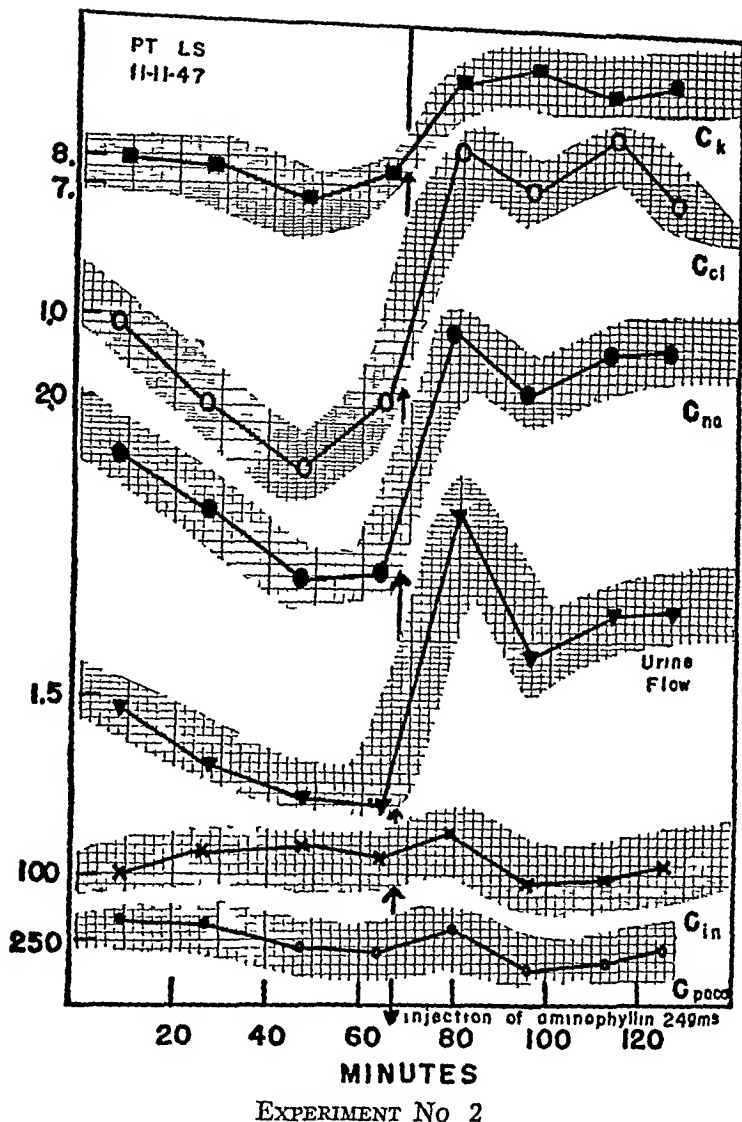
Electrolyte excretion is expressed as plasma clearance (cc per minute)

arth, McMichael and Sharpey-Schaffer (18) found a lowering of venous pressure and increased cardiac output in experiments performed under similar circumstances. Superimposed on the circulatory effects, there was marked increase in sodium, chloride and water output due to decreased tubular reabsorption, which persisted after the changes in the renal circulation had returned to their original normal value. It is interesting to observe that the tubular mechanism initiates the increased excretion of sodium, chloride and water since this change is observed in the first collection period following the injection of aminophyllin (Experiment No 1), at a time when the sodium and chloride clearances had increased three fold while little increase is seen in either filtration rate or renal plasma flow. In the second collection period, this increase in urinary excretion is the summation of both circulatory and renal tubular changes.

Experiments No 2 and 3 show that when the patient was fully digitalized, intravenous aminophyllin caused little or no change in glomerular filtration or renal blood flow. The same three fold increase in sodium, chloride and water excretion produced a diuresis, due to depressed percentage tubular reabsorption of filtered sodium.

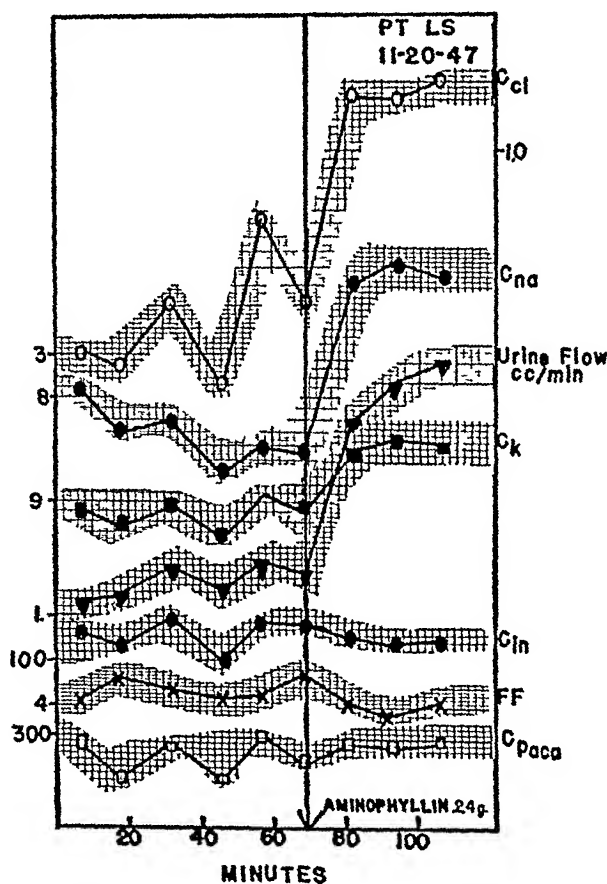
The action of aminophyllin as a diuretic in cardiac decompensation apparently is due to two mechanisms: (1) A direct effect on the circulation, causing a lowering of venous pressure and increased cardiac output which augments glomerular filtration and renal blood flow, (2) a specific renal tubular effect, diminishing tubular reabsorption. Following cardiac compensation there may be cardiac effects (as seen by slight lowering of venous pressure), but there are no corresponding changes in the renal circulation, and the diuresis which ensues is due solely to the action of the drug on the renal tubules.

From these experiments it would appear that the mechanism of action of therapeutic doses of aminophyllin cannot be assessed without consideration of the changes in the physiological characteristics of the patient's disease. The effect of the drug may be upon the whole cardiovascular system as well as directly upon the kidney. The size of the dose and the route and speed of administration may also be important factors in determining the magnitude of the response and whether the predominant response is either cardiac or renal in origin.



*Patient No 1 L S*

Experiment performed after four days of treatment. Four control periods were obtained before the intravenous administration of 0.24 gm aminophyllin. In contrast to Experiment No 1, there are no significant changes in renal hemodynamics following the injection and no alteration in venous pressure occurred. However, the sodium and chloride excretion and urine flow show three-fold increases, a change which continues until the end of the experiment. The magnitude of the increase is interesting in that it corresponds to that seen in the first collection period after the injection of aminophyllin in Experiment No 1. The action of aminophyllin in this instance was entirely on the renal tubular mechanism, causing no alteration in renal hemodynamics. Venous pressure at beginning of experiment was 130 mm water. Electrolyte excretion is expressed as plasma clearance (cc per minute).



#### Patient No 1 L S

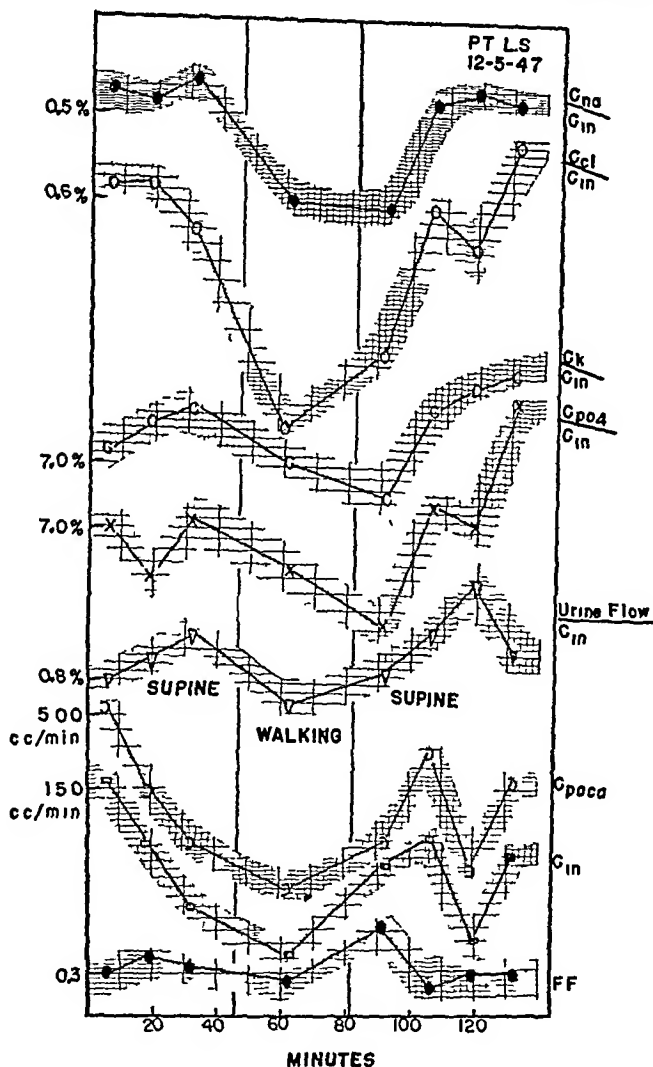
Experiment performed nine days after Experiment No 2 and 13 days after Experiment No 1. Treatment had continued and there had been further clinical improvement although patient still had some pitting edema of the ankles and the liver was still enlarged. Venous pressure was 100 mm water. Six control observations were made before injection. Results obtained in this experiment are similar to those in Experiment No 2. No renal hemodynamic changes occurred following the intravenous administration of 0.24 gm aminophyllin, and the same three-fold increase in sodium, chloride excretion and urine flow occurred. Following the injection, the venous pressure fell to 65 mm water.

Electrolyte excretion is expressed as plasma clearance (cc per minute)

#### *Effect of Exercise in Congestive Cardiac Failure*

Experiments No 4-11 show the changes in electrolyte excretion during mild muscular exercise in Patients No 1, 2 and 3. In all ex-

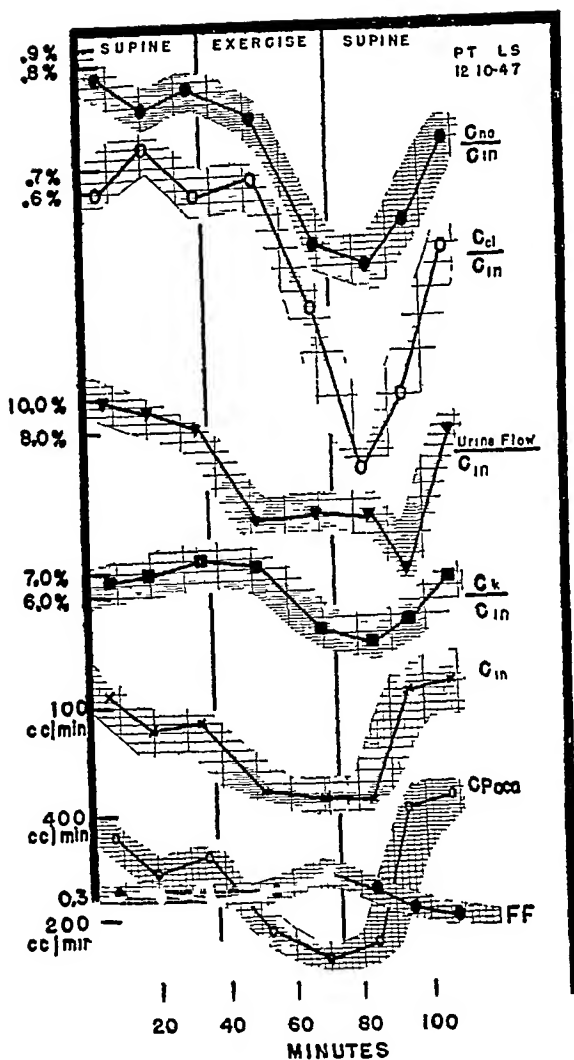




EXPERIMENT No 4

*Patient No 1 L S*

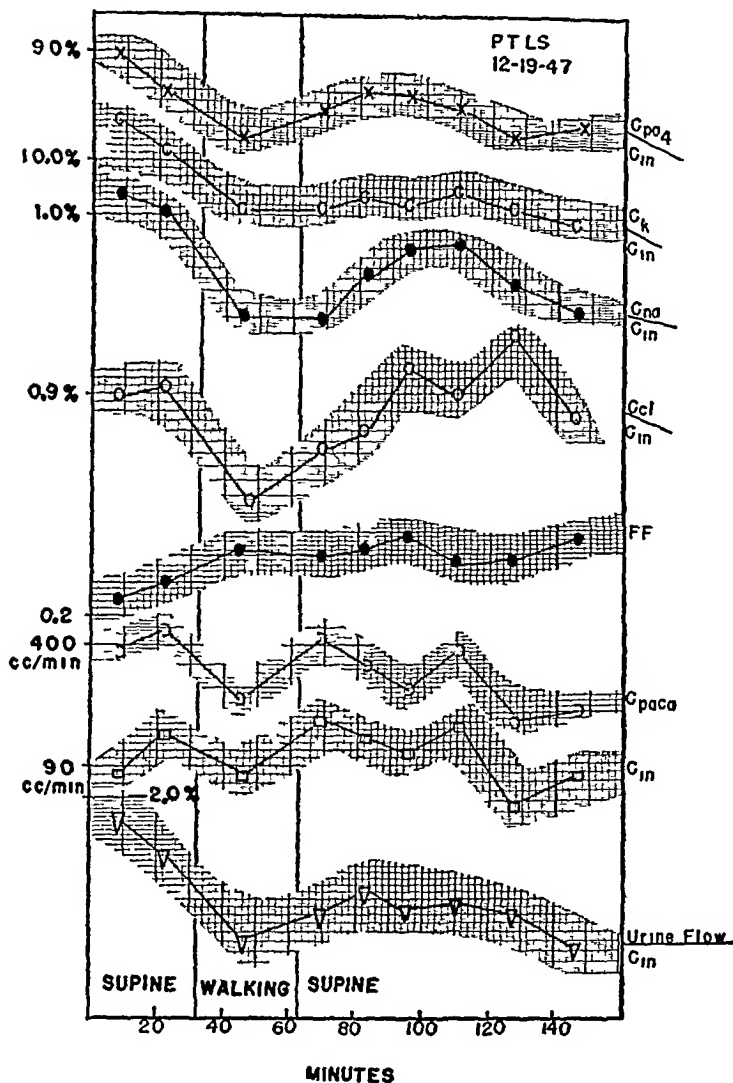
Three control periods in the supine position demonstrate a marked downward trend in the figures for filtration rate and renal plasma, making deductions concerning the effect of exercise on these functions invalid. This was thought to be due to faulty urine collection. The electrolyte excretion ratios are constant prior to exercise, the fluctuations produced by faulty urine collection being eliminated by the method of representation. Exercise (intermittent walking 300 yards in 35 minutes) caused a marked depression in the urinary excretion of chloride and sodium, the latter effect continuing for 23 minutes before  $C_{Na}/C_{in}$  returned to its pre-exercise control level. Venous pressure before exercise was 50 mm water, after exercise, 45 mm water.



EXPERIMENT No 5

*Patient No 1 L S*

The three control periods prior to exercise show a constant filtration rate and renal plasma flow. Exercise in this experiment consisted of 35 minutes intermittent walking along the ward corridor (350 yards). In the first exercise period, an equivalent reduction in renal plasma flow and filtration rate occurs (constant filtration fraction) while the ratios  $C_{Na}/C_{In}$  and  $C_{Cl}/C_{In}$  show no great change. By the end of the exercise period there is further reduction in filtration rate and renal plasma flow, the maximal depression of the sodium and chloride ratios being in the first post-exercise period. The renal hemodynamic changes have returned to control values within 19 minutes, but the increased tubular reabsorption of sodium and chloride continued until the end of the experiment. Venous pressure before exercise was 3 mm water, after exercise, 1 mm water.

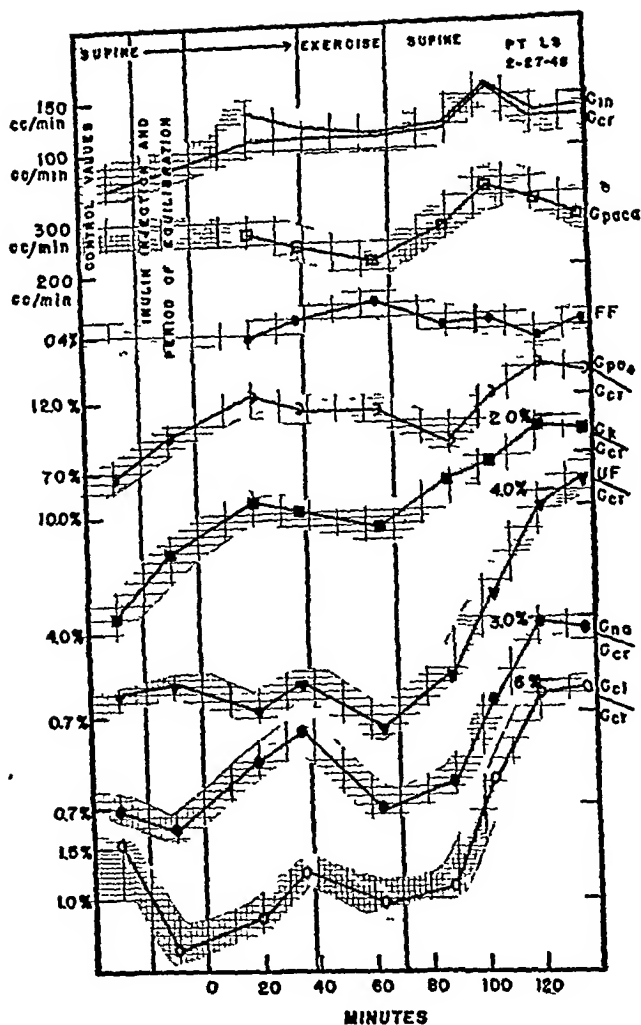


EXPERIMENT No 6

*Patient No 1 L S*

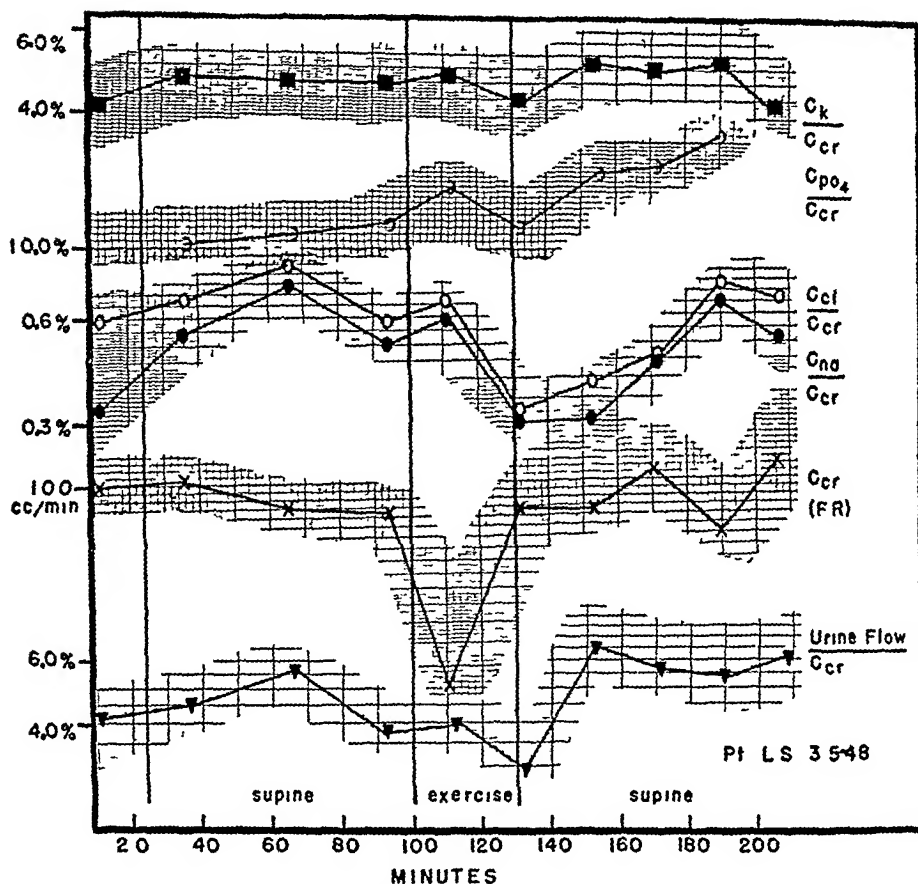
Considerable fluctuation occurred during this experiment in the values for filtration rate and renal plasma flow. The constancy of the filtration fraction would indicate that this is due again to imperfect urine collection. No marked alterations are seen in renal hemodynamics during exercise. Exercise (walking intermittently along ward corridor 400 yards in 27 minutes) produced some anti-diuresis, while a depression in the ratios  $C_{Na}/C_{In}$ ,  $C_{Cl}/C_{In}$  occurred, which in the case of sodium never reached its pre-exercise control level.

Venous pressure before exercise was 8 mm water, after exercise, 2 mm water



#### Patient No 1 L S

Experiment performed during period of supervision in the out-patient department. It will be seen that apart from the UF and Cl ratios, all electrolyte excretion ratios show a progressive rise from the initial control to the pre-exercise values. Exercise (walking intermittently along ward corridor 300 yards for 30 minutes) caused no change in filtration rate but a depression in renal plasma flow and the excretion of sodium and chloride. The mild antidiuretic effect of exercise was followed by increases in filtration rate, renal plasma flow and a four-fold change in urine volume. The effect of exercise in this experiment has been interpreted as a modification of the electrolyte excretion during the course of a spontaneous diuresis, thereby cutting a segment out of the progressively rising curves of all the clearance ratios. Venous pressure, before exercise was 115 mm water, after exercise, 110 mm water.

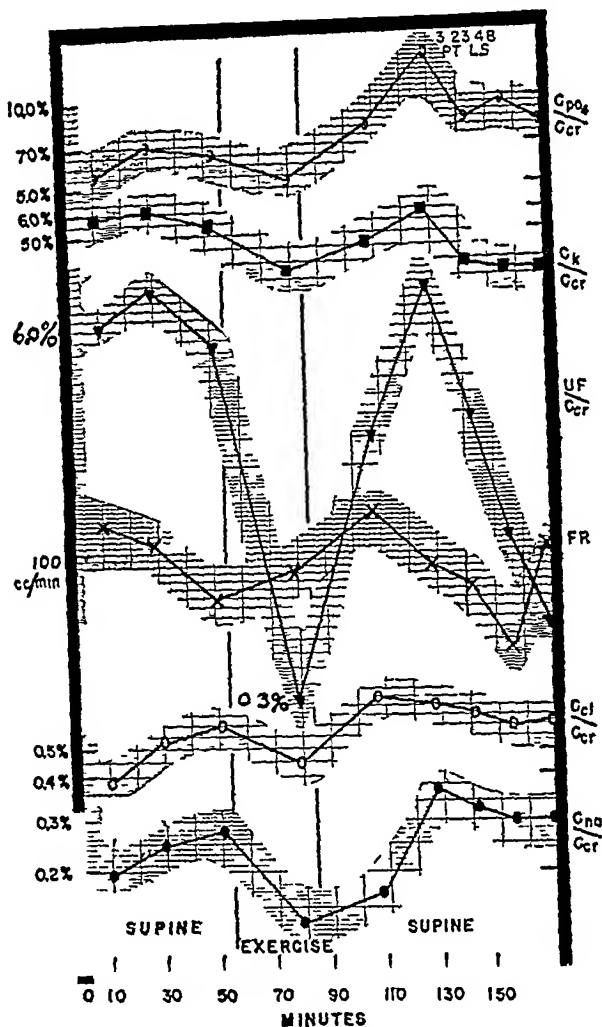


EXPERIMENT No 8

*Patient No 1 L S*

Second experiment performed during period of supervision in the out-patient department. Exercise comprised two eight-minute periods of quiet walking (total of 350 yards) along ward corridor separated by a ten minute standing rest period. Exercise caused an immediate fall in glomerular filtration, while the sodium and chloride ratios remained the same. Return of filtration rate to normal in the first post-exercise collection period was associated with depression of sodium and chloride excretion which returned to pre-exercise level 60 minutes after the end of walking. The clinical condition of the patient at the time of the experiment was not as good as in Experiment No 7. Venous pressure before exercise was 185 mm water, after exercise, 180 mm water.

periments, exercise produced a constant and significant depression in the excretion of sodium and chloride, an effect which continued for an average of 40 minutes after the end of exercise. Filtration rate was depressed during exercise in Experiments No 5, 8 and 11, showed

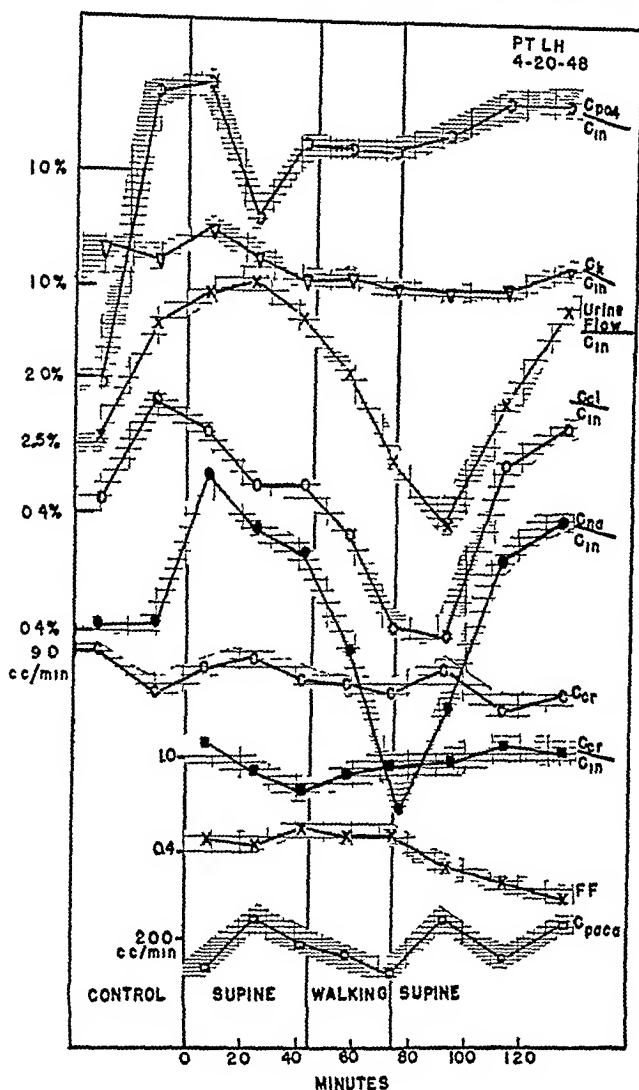


EXPERIMENT NO 9

*Patient No 1 L S*

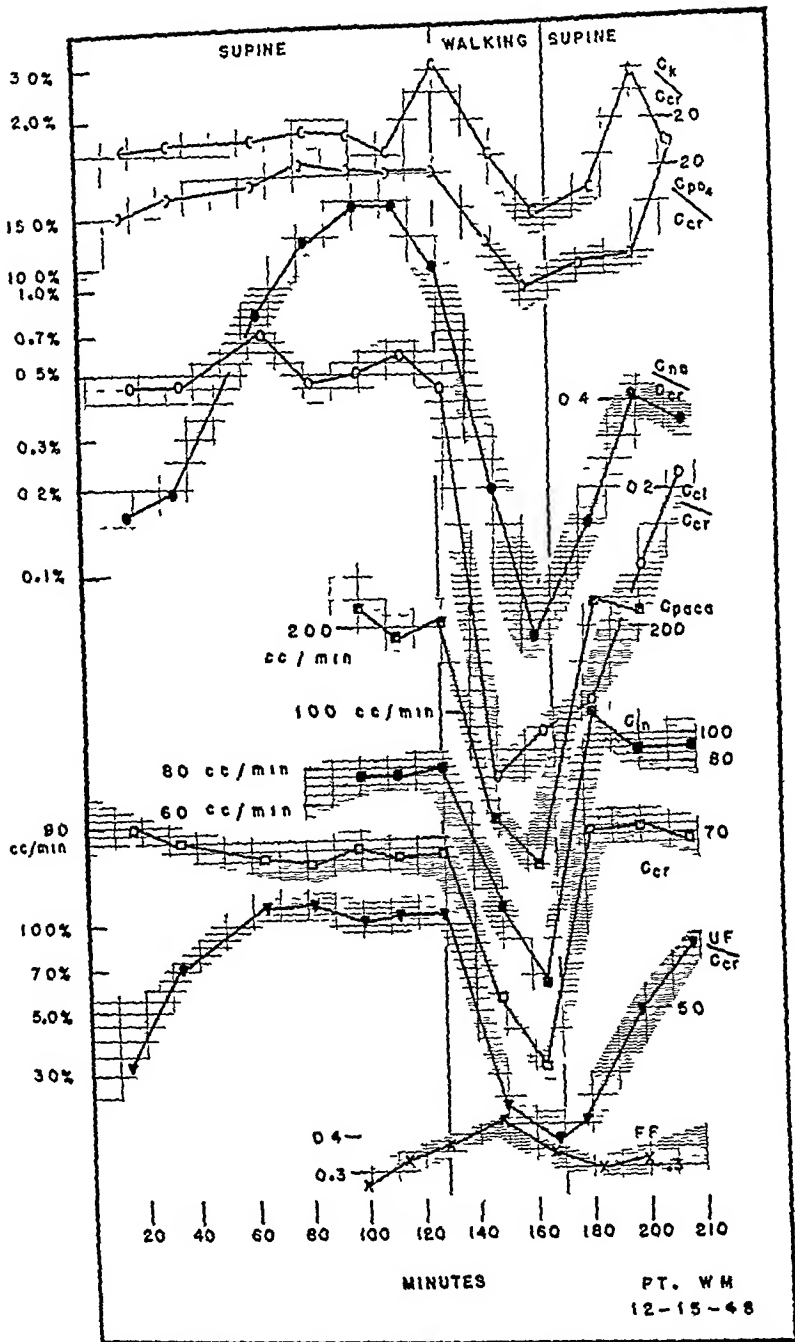
Third experiment performed during period of out-patient supervision. Exercise consisted of walking intermittently 300 yards in 30 minutes along ward corridor. Considerable variation is seen in the filtration rate throughout the entire experiment, due possibly, in this instance, to faulty urine collection. Exercise produced a marked antidiuresis, with depression of the  $C_{Na}/C_{Cr}$  and  $C_{Cl}/C_{Cr}$  ratios. Decreased excretion of sodium and chloride continued for a further 35 minutes after the end of exercise. Venous pressure before exercise was 118 mm water, after exercise, 113 mm water.

no significant change in Experiments No 6, 7 and 10, while no deductions could be made in Experiments No 4 and 9 due to variations in



### Patient No 2 L H

Control period represents 27 minute control urine collection plus one hour for injection and equilibration of inulin (10 gm) and PACA (3 gm). The three collection periods prior to exercise show constant filtration rate and fall in  $C_{Na}/C_{Cr}$  and  $C_{Cl}/C_{Cr}$  to initial control level, following the injection of the test substances. Exercise (walking 400 yards intermittently along ward corridor in 30 minutes) caused no alteration in filtration rate but produced a marked depression of sodium and chloride excretion. This effect is not abolished until the next two collection periods. Venous pressure before exercise was 87 mm water, after exercise, 82 mm water.



EXPERIMENT NO 11

Patient No 3 B M

Seven control periods were obtained prior to exercise, 10 gm of inulin and 3 gm of PACA were injected at the end of the second urine collection period. Apart from the elevation of  $C_{Na}/C_{Cr}$  due to the injection of the test substances, filtration rate, renal plasma flow and all electrolyte clearance ratio are constant. During exercise (walking on a motor driven treadmill for 40 minutes at 1/2 mph), filtration rate and renal plasma flow changed approximately six fold. An antidiuresis was associated with a fall of the ratio  $C_{Na}/C_{Cr}$  from 1.15% to 0.062% which returned to 0.4% 30 minutes after the end of exercise. Congestive cardiac failure in this patient was present with an average filtration rate of 75 cc/min and a filtration fraction of 0.32.



the control periods As the electrolyte clearance ratios depict changes caused only by variations in the tubular reabsorptive mechanism over and above that due to altered filtration rate, the depression of the ratios  $C_{Na}/C_{In}$  or  $C_{Na}/C_{Cr}$  and  $C_{Cl}/C_{In}$  or  $C_{Cl}/C_{Cr}$  are due in these experiments to an increased tubular reabsorption of sodium and chloride This response is similar to that seen in normal subjects, although the degree of exercise in the cardiac patient was less severe than that used for normal controls (15)

#### DISCUSSION

Evidence has been presented in this paper showing the influence of the tubular mechanism in the control of sodium excretion in patients with congestive heart failure Increased excretion of sodium followed the intravenous administration of aminophyllin, while retention was observed during mild muscular exercise These opposing changes have occurred while the filtration rate remained constant, thus demonstrating the lability of the renal tubular mechanism in modifying the electrolyte pattern of the urine This added tubular effect may be an important factor in the retention of salt and water in cases of congestive cardiac failure and differs from the views of Mokotoff, Ross and Leiter (12) who postulated a constancy in the sodium reabsorptive mechanism Retention of sodium as stated by these authors is due largely to a reduction in the absolute amount of sodium filtered through the glomeruli, the cause of such a reduction being a depression of glomerular filtration Mokotoff, Ross and Leiter demonstrated a linear relationship between the amount of sodium reabsorbed per minute and the glomerular filtration rate and found that in their control and cardiac group "despite the variations in the absolute quantities of sodium filtered, reabsorbed and excreted a relatively constant fraction of the sodium in the glomerular filtrate is reabsorbed This amount to a mean of 13.3 mm per 100 cc of glomerular filtrate" Analysis of our findings for the amount of sodium filtered, reabsorbed and excreted in the above experiments yields a figure of similar constancy The constancy of this figure is of little significance because the amount of sodium excreted ( $mEq/min$ ) under any circumstance is of such a small magnitude when compared with the quantity reabsorbed that an apparent or relatively linear relationship between the sodium reabsorbed and the glomerular filtration rate is inevitable

It may be questioned whether the interpretation of these authors represents the most revealing analysis of the data since it places emphasis solely on the variations in glomerular filtration rate with a fixed fraction of the filtrate reabsorbed in order to account for changing urinary sodium excretion. By expressing the urinary output of sodium as a clearance ratio ( $C_{Na}/C_{In} \times 100$ ) changes in sodium excretion which are primarily due to variations in filtration rate have been eliminated. Under such a circumstance (if the changes in excretion of sodium are due only to variations in filtration rate with a fixed proportion reabsorbed) the ratio  $C_{Na}/C_{In} \times 100$  would remain constant. Striking variations in this ratio have occurred in the experiments reported in this paper, demonstrating that the tubular cells play an active part in the economy of salt, either by an increased (see during muscular exercise) or a decreased percentage reabsorption (aminophyllin effect) of sodium in the glomerular filtrate.

A specific salt-retaining response to exercise appears to be a phenomenon common to both normal and cardiac subjects. Whether this response is elicited more readily and more intensely in cardiac patients requires further investigation. During various forms of mild exercise, Merrill and Cargill (19) found that six out of 10 cardiac subjects reacted with a reduction of filtration rate which approached or fell below a critical level of 70 cc/min. These authors state that below this level of filtration patients with a "normal tubular reabsorption of sodium retain salt and water when placed on an average salt intake." Mild exercise in the three cases of congestive failure reported in this paper produced varying results on the filtration rate, as the same individual (Patient L S) on different days showed either a reduction or no alteration in filtration rate. However, on all occasions a marked salt retaining response was elicited. As Merrill and Cargill point out, the limited amount of exercise used in the experimental method may be insufficient to tax the cardiac reserve of these patients and thus bring about detectable alterations either in renal plasma flow or filtration rate.

In 1944 Warren and Stead (10) reemphasized the concept of "forward failure" in the pathogenesis of peripheral cardiac edema, it being suggested that increased plasma volume as resulted from abnormal renal function. Confirmatory evidence was reported by Merrill (11) who showed a reduction in renal plasma flow proportionately greater

than the reduction in cardiac output in cases of congestive cardiac failure. It has been stated that salt and water retention occurs with filtration rates below 70 cc/min (19).

Our results obtained from patient L S indicate that marked congestive failure can occur with a filtration rate within normal limits, although this patient had a reduction of renal blood flow sufficient to produce a filtration fraction of 0.54. Clinical improvement under digitalis, salt restriction and bed rest reduced this value to 0.23. However, with a filtration rate of 101 cc/min and a plasma sodium clearance of 1.02 cc/min when first determined at bed rest, this patient should have been able to maintain a normal sodium equilibrium. We infer, therefore, that the effect of daily activity with its salt-retaining response was responsible, in part, for her edematous condition. The hemodynamic change which seems to be correlated with salt retention is the high filtration fraction which in turn may influence tubular reabsorption. During Period No. 3 figures for sodium clearance show a progressive fall during a period of clinical deterioration. The filtration rate during this period did not show any depression from its previous values, while one determination of the filtration fraction showed a value of 0.49.

The present evidence suggests that if the mechanism of peripheral edema in congestive cardiac failure can be explained by the renal retention of sodium, the sequence of events can best be pictured in the following manner:

Firstly, in patients with a deficient cardiac output under stress, mild activity elicits more readily and intensely a salt-retaining response similar to that seen in normal subjects. Secondly, owing to a deficiency in cardiac output, less blood is diverted to the kidney. At this stage, through constriction of the efferent glomerular arterioles, glomerular filtration proceeds at a normal rate. Any retention of sodium and water at this stage would still be due to the increased tubular reabsorption of this electrolyte. Thirdly, in more advanced stages of cardiac decompensation and with greater stress, a reduction in glomerular filtration rate occurs, causing an added retention of sodium owing to a diminution in the total amount of sodium which is filtered through the glomeruli. That such a tubular effect does occur during the life of the ambulant cardiac patient is shown by the experi-

ments reported in this paper. We agree that it is probable that "altered reabsorptive faculties of the tubules precede the extravasation phenomenon at the peripheral capillary membrane" (20).

The mechanism of this salt-retaining response to muscular exercise has been considered elsewhere (15). It would appear not to be mediated by the posterior pituitary gland, although the antidiuretic effect of exercise has been shown by Verney (21) to be due to humoral influences of this gland. Smith and McKay (22) have demonstrated in normal subjects that the antidiuretic principle of the posterior pituitary causes an increase in the excretion of sodium and chloride with resulting negative balances of these elements. This effect is different from that which is observed during muscular exercise.

The known importance of the adrenal glands in the control of sodium excretion immediately suggests further mechanisms, but little is known of adreno-cortical activity in congestive cardiac failure at the present moment.

It has been emphasized that the excretory function of the kidney should be more closely allied to extracellular fluid volume (23), since the chemical anatomy of that compartment is controlled largely by renal mechanisms. Assuming constant renal function, the larger the extracellular fluid volume, the greater is the time required to clear a known amount of electrolyte from this space, because the electrolyte will be dispersed in low concentration through a large volume of fluid. Such delay in excretion of ingested sodium has been demonstrated on clinical grounds (8) and by the use of the radioactive element (9). Retention of sodium in an already edematous patient carries with it the inherent possibilities of progressive accumulation when viewed in the light of the theoretical relationships which exist between the various fluid compartments and the ability of the kidney to clear any electrolyte from the body. The fate of the edematous cardiac patient is closely allied with the ability of the kidney to maintain salt and water balance, to which end the renal tubular cells appear to play no insignificant role. In the words of Starling, "We see, then, that the production of dropsy in heart disease is by no means so simple as it first appears, but that it is due to a complicating series of interacting mechanisms all of which tend towards the death of the organism."

## SUMMARY

1) Observations are reported on the effect of intravenous aminophyllin, mild muscular exercise and rest on the renal mechanism of electrolyte excretion in cases of congestive cardiac failure

2) Electrolyte excretion has been expressed mainly by clearance ratios (the ratio of the clearance of electrolyte to the clearance of inulin or creatinine  $\times 100$ ) which records changes in the renal tubular reabsorption of electrolyte in terms of the filtered electrolyte not reabsorbed by the tubules

3) Observations on one patient extended over a period of four months, including metabolic balance studies of sodium, chloride, potassium and nitrogen which were carried out for twenty-four days

a) This case had a glomerular filtration rate within normal limits with a reduction in renal plasma flow and a high filtration fraction when first determined. With weight loss, diminution in peripheral venous pressure and sodium chloride loss under digitalis, bed rest and salt restriction, the filtration rate remained within normal limits, the renal plasma flow increased and the filtration fraction decreased to normal values

b) The patient showed a positive nitrogen and potassium metabolic balance during the loss of sodium, chloride and weight. The amount of potassium retained was more than would ordinarily be expected due to retention of nitrogen. This suggests that disturbances in intracellular electrolyte and nitrogen structure may occur in congestive cardiac failure, reflecting an injury to the body cells and should be further investigated

4) Mild exercise in three cases of congestive cardiac failure evoked a sodium chloride retaining response, characterized by a decrease in the fraction of filtered sodium not reabsorbed by the renal tubules (clearance ratio). Exercise was sometimes accompanied by decreases in glomerular filtration rate, but constantly resulted in a decrease in the sodium clearance ratio. The decrease in sodium excreted persisted after return of filtration rate to normal or to its pre-exercise level

5) The action of aminophyllin on the kidney in cardiac decompensation is due to two mechanisms and is modified by the degree of decompensation

(a) A direct effect on the circulation, causing augmentation of glomerular filtration rate and renal plasma flow presumably due to increased cardiac output

(b) A specific renal tubular effect, diminishing reabsorption of filtered sodium

6) Speculation is made concerning the role of the salt retaining response of exercise in the pathogenesis of peripheral edema in congestive cardiac failure

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# MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

MONDAY, JANUARY 10, 1949, AT 8 15 P M

HURD HALL, THE JOHNS HOPKINS HOSPITAL

*Dr Follis* I would like to open this, the third meeting of the Johns Hopkins Medical Society for this year The first speaker will be Dr C L Conley

## AMINOPTERIN IN THE TREATMENT OF ACUTE LEUKEMIA

Since April, 1948, nine consecutive cases of acute leukemia have been treated with aminopterin (4-amino pteroylglutamic acid) Three of the patients were children and six were adults Two cases were classified as acute lymphogenous leukemia, the remainder as acute myelogenous Two patients showed no response to the drug, either clinically or hematologically, except for the development of stomatitis The others showed a prompt fall in the white count, at times to extreme leukopenic levels In no case did abnormal cells disappear from the blood, nor did the marrow alter from a leukemic pattern One patient developed cells resembling megaloblasts in the marrow after 3 weeks of therapy Several patients displayed transient reduction in the size of lymph nodes and spleen, but in only one patient was there a rise in hematocrit and platelets during therapy No striking clinical remission was observed Toxic manifestations were common and included stomatitis, diarrhea with bloody stools, and severe depression of the marrow Individual patients showed marked variation in their tolerance to the drug Six of the 9 patients are dead Autopsies on 3 patients showed no pathologic changes which could definitely be attributed to the therapy

*Dr Follis* Dr Conley's paper is now open for discussion

*Dr Schoenbach* I think it is indeed a privilege to hear someone of Dr Conley's capabilities discuss this subject There are a few matters I would like to have clarified Of these cases studied, there is one which was not included It was a case of acute leukemia The child who was treated was acutely ill and there was marked bone change The child showed a remarkable response and at the present time there is question whether he has leukemia at all I would like to know why, several months later, that case was not included in a series of consecutive cases

The term "antifolic acid compound" was used throughout There is no evidence that its action, either versus neoplasms or leukemia, can be directly attributed to its antifolic acid activity The compound has antifolic acid properties, but it also has been shown to inhibit the action of other essential metabolites as well as other steroid hormones

I think we need far more critical study of these leukemic diseases We should have more objective criteria for classification based more on function than on morphology Perhaps the chemist can then tell us whether he can inhibit that metabolic function, or process This may enable one to select the cases which will respond



Aminopterin is a toxic compound. How much of the toxicity is due to its specific action and how much is non-specific one cannot evaluate until its pharmacology is known.

The third most important fact about toxicity is that we are looking for a differential effect upon neoplastic and normal cells. Animals are reproducible to a certain degree as to age, weight, sex, etc. One can inoculate them with a tumor whose biology is known and one can achieve remarkable histologic and developmental changes with doses of aminopterin far removed from the toxic effects.

I think the problem should really remain at that point for a long period of time, because if one could find what metabolic process has now been inhibited one would have a clue as to the necessary metabolic function in the neoplastic cell which apparently is not required by the normal cell. With that clue in mind, we would have some knowledge of what mode of approach to take in the development of future chemical compounds, which are far more active and far less toxic.

I think we should look at these patients who have received aminopterin as an extension of basic laboratory investigations. Certainly 4-aminofolic acid, and closely related compounds, are not yet chemotherapeutic agents for the treatment of leukemia.

*Dr Conley* Of two patients omitted, one was a case of Hodgkin's disease and we presented here only cases of acute leukemia, and the other was a seven-year-old boy who showed many symptoms of acute leukemia but never presented a leukemoid blood picture. Several attempts at biopsy failed to produce satisfactory material, so diagnosis was never established.

*Dr Schoenbach* Were the biopsies all made after treatment?

*Dr Conley* Yes.

*Dr Schoenbach* You performed an aspiration before treatment, which was typical of acute leukemia.

*Dr Follis* Is there any further discussion of Dr Conley's paper?

If not, we will go on to the second paper on tonight's program by Drs R J Bing, M Hammond, J Handelsman, S Powers and F Spencer. Dr Bing will present the paper.

## CORONARY BLOOD FLOW, CARDIAC OXYGEN CONSUMPTION AND CARDIAC EFFICIENCY IN MAN

The work reported is the outgrowth of the chance observation that the coronary sinus of man can be intubated through the right auricle. An attempt was consequently made to utilize this technique for the measurement of coronary blood flow in man. In order to accomplish this, it was decided to embark on a joint cooperative program with Dr Eckenhoff and his associates from the Department of Pharmacology in Philadelphia and with Dr Goodale of the Army Chemical Center at Edgewood, Maryland. These two groups of investigators had developed a procedure for the determination of coronary blood flow in the dog, combining the use of the nitrous oxide method with that of catheterization of the coronary sinus. The

nitrous oxide method, originally developed by Kety and Schmidt for the determination of cerebral blood flow, is applicable in principal to any organ from which a representative sample of venous blood can be obtained. The heart by virtue of its homogeneous structure and its venous drainage into the coronary sinus is particularly suitable. The coronary blood flow obtained with the nitrous oxide method represents the cc's of blood coursing through 100 grams of left ventricular muscle in one minute. By multiplying this volume by the difference in the oxygen content between arterial and coronary sinus blood, one obtains the oxygen content of 100 grams of left ventricular tissue. The results obtained per 100 grams of cardiac tissue may be considered accurate. It should be emphasized, however, that values for left ventricular oxygen consumption and efficiency are approximations, since the weight of left ventricular muscle can be obtained only indirectly. It seems justified to estimate the left ventricular efficiency of the normal heart, since its weight can be obtained from available tables with a reasonable degree of accuracy. It is impossible, however, to calculate left ventricular efficiency in abnormal hearts, since the degree of hypertrophy cannot be determined. The calculation of left ventricular efficiency, using normal weights, will result in maximal values. Consequently, the finding of decreased efficiency is of significance, since the true efficiency must be even lower. This is the case in cardiac failure.

The studies were performed on normal individuals and on patients with anemia, coarctation of the aorta, essential hypertension, and heart failure due to mitral stenosis and insufficiency. In addition, two patients with aortic stenosis and insufficiency respectively were studied. Only 50 per cent of all attempted catheterizations of the coronary sinus were successful. This was probably due to anatomical variations in the structure of the right auricle, for instance, differences in the height of the Eustachian ridge.

The results showed that in normal man the coronary blood flow averaged 65 cc per minute per 100 grams of cardiac tissue. The arteriovenous oxygen difference was 12 volumes per cent, and the oxygen consumption 7.8 cc per 100 grams per minute. In anemia the coronary flow was increased, the arteriovenous oxygen difference and the oxygen consumption were reduced. In essential hypertension, mean values for coronary blood flow, the arteriovenous oxygen difference, and the oxygen consumption were normal (mean values 70 cc/min/100 grams, 10.6 vol per cent, and 7.4 cc/100 grams/minute). In coarctation the coronary flow, the arteriovenous oxygen difference, and the oxygen consumption were markedly increased (mean values 83 cc/grams/min, 14 vols per cent, and 11.6 cc/100 gms/min). In cardiac failure due to mitral stenosis and insufficiency the coronary flow was slightly reduced to a mean value of 60 cc/100 gms/minute, but the arteriovenous oxygen difference was increased to a mean value of 14.4 vols per cent. The oxygen consumption per 100 grams of left ventricular tissue was slightly increased (8.6 cc/100 gms/min). In the patients with aortic insufficiency the coronary blood flow and the cardiac oxygen consumption were increased. In contrast, in the patient with aortic stenosis the coronary blood flow and the cardiac oxygen consumption were within normal limits.

The per cent efficiency of normal left ventricular muscle (the ratio of left ventricular work and energy cost expressed in kilogram meters) averages 23 per cent. In failure due to mitral stenosis and insufficiency, the calculated maximal left ventricular efficiency is decreased. Consequently, true values must be even lower.

*Dr Follis* Dr Bing and his collaborators' paper is now open for discussion.

*Dr Andrus* These results open up a very significant advance in the study of heart disease in man. As Dr Bing has mentioned, the results obtained from studies made on the heart-lung preparation, are deficient in very important respects though they have undoubtedly contributed a great deal to the understanding of cardiac physiology. But the road toward discovery has been littered with disputes as to the significance of results which have been obtained with relation to cardiac failure.

The situation with regard to the understanding of heart failure, as attempts have been made to derive this from studies on the heart-lung preparation, is comparable to looking at a baseball game through a knothole in the fence. Conclusions depend a great deal upon which way the knothole points and which part of the field it is possible to view through it.

Dr Bing has quite properly mentioned certain assumptions. I would like to have two of these clarified before I have entirely comfortable feelings about the results. The first has been the basis of some of the disputes regarding the mechanism of failure in the heart-lung preparation. Only about sixty per cent of the blood which enters the coronary arteries comes out through the coronary sinus. The remainder penetrates to the cavity of the ventricles through Thebesian channels which do not communicate with the coronary sinus. What is there about the nitrous oxide method that makes it possible to be certain that the catheter is getting a representative sample of the total coronary flow?

Another assumption mentioned that the volume, or the weight, of cardiac muscle involved in the normal and in the failing heart is the same, of course, a very important one. If I understand the figures correctly, the assumption that the cardiac weight was increased in hearts with failure would only add to the significance of data in the same direction in which Dr Bing has interpreted them.

I can't close without expressing my admiration for the energy and the enthusiasm and the patience which has been applied to this study. I hope that as he casts toward the coronary sinus Dr Bing may get a higher percentage of strikes in the future.

*Dr Follis* Is there other discussion, or any other questions?

*Dr Schoenbach* There is one question I would like to ask which is essentially mathematical. There is an integral with limits, zero to "U", which is not explained. I would like to know what the zero to "U" was and how one derives that integral. I only saw the four points on the curve.

*Dr Follis* Is there any further discussion?

*Dr Rich* May I ask Dr Bing a question which is in line with one of the questions that Dr Andrus asked? I was most interested in the difference between the

coronary blood flow in heart failure in coarctation of the aorta and hypertension because so much depends there on this hundred grams I wonder if Dr Bing would tell us a little as to how he arrives at how many hundred grams there are

*Dr Marshall* I would like to ask Dr Bing, out of curiosity, how he determines the solubility of the nitrous oxide molecule

*Dr Follis* Is there any further discussion of Dr Bing's paper?

*Dr Bing* Dr Andrus brings up the important question whether or not coronary sinus blood represents true mixed coronary vein blood The question should be answered in the negative The coronary sinus carries only blood which has perfused that part of the cardiac muscle which drains into the coronary sinus Other portions of heart muscle may have a different oxygen extraction Therefore, by means of the nitrous oxide method, we measure only the coronary blood flow and the oxygen consumption of that portion of the heart from which blood drains into the coronary sinus This probably represents left ventricular muscle The nitrous oxide method for the measurement of coronary blood flow would be invalidated if the coronary sinus contained mixed venous blood However, such contamination can be suspected if the arterial and venous nitrous oxide concentrations fail to come to an equilibrium at the end of eight minutes

Dr Rich has put his finger on another weak spot when he asks how many hundred grams there are per total ventricular muscle We calculate coronary blood flow and oxygen consumption per unit weight, since  $S$ , the partition coefficient, represents the ratio of nitrous oxide dissolved per gram of heart muscle to the amount dissolved in cc of blood at a constant nitrous oxide tension and at 37 degrees No assumptions are necessary in these calculations However, in order to estimate how many hundred grams there are per left ventricular tissue, assumptions have to be made There are several possible methods for the estimation of the weight of the left ventricle One may estimate cardiac weight from x-ray data, using the anterior-posterior and lateral position We found this unsatisfactory We are now using the tables of Smith, who from several hundred autopsies related body weight to the minimal and maximal heart weight in normal individuals It is obvious that such a table can only give approximate figures The left ventricular weight is assumed to constitute 53 per cent of the total heart weight As I said before, we omitted calculations of cardiac efficiency in abnormal hearts But we have reasons to believe that the left ventricular efficiency in failure is low

Concerning Dr Marshall's question on the determination of the solubility of nitrous oxide in heart muscle Eckenhoff and his associates used the procedure outlined by Kety and his co workers for brain They placed dog or human heart homogenized in a Waring blender in a tonometer and allowed it to come into equilibrium with nitrous oxide The tissues were then analyzed for nitrous oxide in the Van Slyke apparatus

To answer Dr Schoenbach's question When the nitrous oxide analyses are completed the values are plotted against time The time of each sample is taken as the mid point of the interval for which the sample was taken, except for the first

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To answer Dr Schoenbach's question When the nitrous oxide analyses are completed the values are plotted against time The time of each sample is taken as the mid-point of the interval for which the sample was taken, except for the first

pair taken at a constant rate over the first minute (the integrated sample) Smooth curves are then drawn through the arterial and venous points From these curves the values for arteriovenous nitrous oxide and the arterial venous nitrous oxide difference in blood are obtained at the end of each minute over the eight minute period

*Dr Follis* The last paper, by Drs W E Goodwin, R D Sloan, and W W Scott, is entitled "The Trueta Renal-Vascular 'Shunt'" and will be presented by Dr Goodwin

### THE "TRUETA" RENAL VASCULAR "SHUNT"\*

#### AN EXPERIMENTAL DEMONSTRATION OF NEUROVASCULAR CONTROL OF THE RENAL CIRCULATION IN THE RABBIT, CAT, DOG, AND MONKEY

Trueta's concept of a renal vascular "shunt" mediated by the sympathetic nervous system was discussed, and some of the literature and early work pertaining to the subject was reviewed briefly

The methods and results of experiments designed to study this concept in the kidneys of 40 rabbits, 40 dogs, 4 cats, and 4 monkeys were presented The results of these experiments except in one case failed to show a reflex control of renal circulation with sciatic stimulation as described by Trueta However, further experiments with direct splanchnic and renal nerve stimulation showed a marked neurovascular effect on the renal circulation with subsequent renal vasoconstriction varying from slight to almost complete ischaemia

In some of the sections it is demonstrated that this ischaemia is first cortical with juxtamedullary glomeruli carrying the bulk of whatever blood is flowing through the kidneys Some of the x-ray studies suggest the existence of cortical ischaemia with an increase of juxtamedullary and medullary concentration of the radio-opaque dye in the stimulated kidneys as compared with the unstimulated control

A brief moving picture in color made by Mr William P Didusch was shown It recorded some of the animal experiments described in the paper

This was considered to be a demonstration of a neurovascular control of the distribution of renal blood flow under the rather extreme and unphysiological conditions of these experiments

*Dr Follis* Dr Goodwin's and his collaborators' paper is now open for discussion

*Student* Dr Goodwin, I believe in Trueta's monograph he mentioned that the blood from these juxtamedullary glomeruli went through the basi recti Is that correct? (Dr Goodwin answered in the affirmative) In these experiments, I didn't notice any of the dye in the basi recti I wonder if you have any explanation for that

*Dr Goodwin* I don't have any explanation for that We looked and looked

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\* To be published in *The Journal of Urology* Vol 61, May 1949

for that, and we never could understand whether it was because there was not enough dye getting in, or whether the time interval was wrong, or what was the difficulty. That was true of the india ink experiments. If done with trypan blue, or methylene blue, instead, which seem to mix better with the blood, you do get a bluing of the medulla with a pale cortex. However, that is apt to be a little confusing, because the controls sometimes look something like the experimental when those dyes were used, so we limited most of these to india ink, as we thought we could see it better under the microscope.

*Dr Grafflin* I should like to emphasize that while there is a very marked constriction of the glomeruli on the stimulated side in these experiments, the vasa recta do not stand out in a way at all comparable with those illustrated by Trueta and his group. In amplification of Dr Goodwin's brief reference to Spanner's work, Spanner claimed to have demonstrated numerous arteriovenous anastomoses in the renal capsule, pelvis and cortex. Trueta *et al* state that they could not confirm Spanner's observations, although it is not clear how deeply they went into the problem.

*Dr Follis* Is there any further discussion? If not, the meeting is adjourned.



## BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal)

*Pathology* By W A D ANDERSON AND COLLABORATORS, pp 1426, \$15 00 C V Mosby Co , St Louis, 1948

This volume edited by W A D Anderson and 31 collaborators represents the most ambitious work on pathology attempted in this country to date. Because of the multiple authorship the material which is presented varies in quality. Some sections are excellent, while others are not at all impressive. However, each contains a great deal of information documented by an extensive bibliography, especially on pathologic anatomy, pathologic physiology is, in the main, inadequately treated. The book is arranged along traditional lines into General and Special Pathology. Such a scheme not only results in repetition but also fails to present a coherent story of disease. For instance, if one wishes to read about lymphogenous leukemia, he must turn to a number of sections, each written by a different author. *Blood, Bone Marrow, Spleen, Lymph Nodes*, etc. Perhaps the most objectionable characteristic of this book is its format. Much of the text is in extremely small print, when larger type is utilized it comes as a relief. However, the criteria employed in determining the point size for the subject in question are completely obscure. As a result of the typography, the pages in general are unattractive and make for difficult reading.

Because of its voluminous nature, for there are 1426 pages of text, much of which is in small print, and because of the arrangement of its subject matter, we would not care to recommend this work to the second year student of medicine who is meeting the subject for the first time. Nor would it seem particularly adapted to the needs of those specializing in clinical medicine since the necessary approach to an understanding of the biology of an entire disease is lacking. This volume, however, should be valuable to those few who are specializing in pathology and who, therefore, need a reference work in pathologic anatomy.

R H F, Jr

*Liver Injury Transactions of the Seventh Conference* F W HOFFBAUER, Editor  
New York, The Josiah Macy, Jr Foundation, 1948

This small volume is a transcript of the latest of the stimulating conferences on liver injury held under the auspices of the Josiah Macy, Jr, Foundation. The papers presented were concerned with the physiology of the liver and its alteration under experimental and diseased conditions. The topics discussed included the locus of formation of plasma proteins, the hepatic circulation and its surgical alteration, the regeneration of injured liver tissue, the mechanism and use of various tests of hepatic function, and the pathologic effects of plutonium on the liver.

The use of radioactive isotopes in the study of hepatic physiology is of particular interest. It is to be hoped that all the material presented will soon be available in more readily accessible periodicals.

O D R

*Introduction to Psychobiology and Psychiatry* ESTHER LORING RICHARDS, M D  
ScD *The C V Mosby Company, St Louis*, 1946 Second Edition 419  
pp \$3 75

Dr Richards' book is written for nursing and medical students with the aim of preparing each of these groups so that they become "sufficiently aware of major and minor sweeping emotional illnesses (psychoses and psychoneuroses) so that patients in the early stages of these illnesses can be competently treated or promptly referred elsewhere." Mere print cannot reflect the animation, humor and positiveness that Dr Richards can achieve in her teaching, but there is enough of the flavor of her personality captured in this book to wake a good many active memories of her teaching in her former students.

The attitude toward the patient set forth in this book is indicated by the fact that no patient is referred to by his surname. For the psychiatrist who has come to feel that one of the primary requirements of good rapport is a sincere recognition of the individuality, sensitivity and self-respect of the patient who will, under treatment, become a collaborator in his own therapy, this attitude is disturbing. In child psychiatry, it is perhaps permissible to read, "Grace, a seven year old," but to find, "Miles was a man of fifty-eight," "Laura is a married teacher of thirty-eight," reflects an attitude toward the patient which many therapists feel is inimical to rapport. It is doubtful whether an attitude which places the patient in a dependent, rather lower class than his doctor or nurse is a good one to teach to students, however much it may lend itself to the kind of rapid treatment processes to which an extremely busy out-patient department can force psychiatrists. "Ben is a government employee of thirty-five making \$4200 a year." A government employee with inadequate education but with sufficient force to reach such a salary deserves at least a "Mister" and his treatment for most psychiatrists would demand that his value as such be recognized. Many authors of nursing texts insist that nurses call all patients, particularly psychiatric ones, by their proper title and surname.

The section on psychobiology is designed to reflect Dr Adolf Meyer's concepts. It follows the outline of his personality study form exactly. The patterns of biological development are presented very briefly—the psychobiology of infancy, childhood and adolescence are included from pages 39 to 43. In many areas concepts of constitutional determination of personality reactions are tacitly assumed where more psycho-dynamically minded students see possibilities, at least, of basic psychogenic factors. Dr Richards has little patience with such philosophies of psychiatry, she is primarily concerned with the here and now of the patient's illness and the factors in it that can be dealt with directly and at once. In line with this, the treatment sections which are generally quite short, from less than a page

to no more than two, are concerned exclusively with very direct "advice and counsel"

The book contains some serious inaccuracies. In the section on the treatment of epilepsy this statement appears "Specific drug therapy is confined pretty generally to luminal (phenobarbital) and sodium bromide." To be sure, Dilantin and Tridione are mentioned later on the page but the statement quoted was certainly not true in 1946. It was probably allowed to persist into the new edition as an oversight. A similar oversight is made in the statement that alcohol "acts first as an excitant and second as a depressant." The supporting material is all of the type generally used to sustain the view that alcohol is a depressant to the nervous system. In the appendix there are statements about the projective personality tests that are similarly in error.

To address the same book to students of medicine and student nurses is a most unusual procedure, though probably one that should more often be carried out. It accomplishes a much needed purpose in stressing that in medicine the physician and nurse are a team, each with his own special job to be sure, but each knowing how the other works and thinks.

The real value of Dr. Richards' book is the philosophy of service in medicine it depicts and which is so beautifully illustrated by the life of the author. On page 37 she says, "Doctors and nurses both are up against the most exacting of all occupations—one that taxes without let-up, head and body and heart." There is a real danger that willingness to give the full measure of devotion to the service of mankind may wane as the exact sciences entice the physician to greater objectivity. To physicians like Dr. Richards, what goes on in test tubes will always be only the handmaiden of a practice of medicine concerned with the health of living, feeling human beings.

P V L

*The Basis of Chemotherapy* By THOMAS S. AND ELIZABETH WORK 435 pp  
\$6.50 Interscience Publishers, Inc 215 Fourth Ave New York, N Y

A completely rational chemotherapy of infectious disease is probably some distance in the future for as the authors remark "The problem of reducing drug action to interference with any single enzyme is immense, and the study of intermediary metabolism is likely for the present to do more towards suggesting new types of drugs than towards solving the problem of the mode of action of known drugs." Thus it is, that most successful drugs have been at least partially happenstance, even when a studied attempt was made to find a new drug. The gradual evolution of atabrine from the known effect of methylene blue is such an instance. And yet the very intensive studies of antimalarials and their mode of action, which are nicely summarized in this book, do not explain how or why known antimalarials act.

The last decade or so has witnessed a tremendous application of all sorts of specialized disciplines to the general problem of chemotherapy. The dependence of the typhoid bacillus on tryptophane for growth, the genetics of a mold, neuro-

spora, growth factors for chicks, the formation of adaptive enzymes by bacteria, the toxicity of mercury, the absorption of viruses on cells, have all been fused into the modern "basis for chemotherapy"

This book is an excellent summary for all experimental workers interested in infectious diseases and their treatment. One of its chief merits is the combined consideration of results obtained in the chemotherapy of protozoan diseases with the more rapidly expanding bacterial chemotherapy. A bibliography of some 54 pages is not the least of its virtues.

F B B

*A History of the Heart and the Circulation* By FREDERICK A WILLIUS AND THOMAS J DRY 456 pages, \$8 00 *W B Saunders Company*, 1948

As stated in the Preface, because "the professional historians have been accorded several centuries to fulfill their obligations in this regard but have not as yet produced a comprehensive work" (on this subject matter) the authors feel that this book is justified and that "the interpretation of medical data, even those in remote eras, is in reality a function of the physician." The first quotation here is true beyond question, and there is much truth in the second statement.

The book is divided into three main sections, the first being a chronological presentation of brief biographical sketches of men who made contributions to the knowledge of the heart and circulation. In the second section there are special biographies of the twenty men chosen by the authors as having been the outstanding contributors to this field. The third section then divides the cardiovascular subject matter into eighteen parts and discusses the contributions to each chronologically. Except for the third section, each chapter is followed by a list of references to the outstanding works written by or on the men concerned.

The biographical sketches are written clearly and whereas the major emphasis is on each man's contribution to the cardiovascular field, enough is added about his personality and environment to make for fairly interesting reading. This is particularly true of the second section.

The book's chief virtue lies in its being a fairly comprehensive reference text. Because the authors are physicians and not historians, and because of the brevity of the book, a true picture of the men discussed and the relative value of their contributions has not always been given.

R M

*Report of the Sanitary Commission of Massachusetts 1850* By LEMUEL SHATTUCK AND OTHERS 321 pp, \$4 50 *The Harvard University Press, Cambridge, Massachusetts*

The clarity of vision which is necessary for a great work frequently comes from outside a profession. Such is the case in this magna charta of public health which was published originally almost a century ago, but which nevertheless is fresh and pertinent today. Lemuel Shattuck was primarily a bookseller and publisher, but was also interested in history and genealogy. His interest in the latter awakened

an interest in statistics and from that he was led to a sanitary survey of the city of Boston in 1845. In 1849, as a member of the state legislature he secured the appointment of three commissioners "to prepare and report to the next General Court a plan for a Sanitary Survey of the State." The other members besides himself were Nathaniel P. Banks, Jr. and Jehiel Abbott.

Each reader in going through this document will pick out very different items of interest—so wide is the survey. This reviewer was struck by the accounts of bilious remittant fevers occurring during the summer and fall in areas near mill ponds (pg 74-76). These epidemics many competent people attributed directly to the existence of the dammed up streams by way of noxious effluvia. Descriptions of the epidemic and the situations make it likely that they were dealing with malaria.

A perusal of the mortality tables for Boston and for an interior country town shows clearly how much safer it was to live in the country than in a large city. Under the age of 5, the annual mortality *per cent* was 9.55 for males and 8.44 for females. It was 3.05 for both sexes in the country. Or to put it in the words of the report "In Boston, of those under five years of age, 9 out of every 100 died" in one year.

On page 98, the report points out that consumption "the dreadful disease is a constant visitor to all parts of our Commonwealth," but creates little alarm because it is so constantly present, whereas the occasional visit of cholera or some other epidemic disease creates alarm and, therefore, precautionary measures are taken. How true today that still is.

It is impossible to review each recommendation in the Report. A striking one is XVII "We recommend that in laying out new towns and villages, and in extending those already laid out, ample provision be made for a supply, in purity and abundance, of light, air and water, for drainage and sewerage, for paving, and for cleanliness." The economic waste and lack of sanitation involved in sending untreated sewage out into the rivers is carefully analyzed and it is suggested that methods be worked out to put this to use. Vaccination against smallpox is strongly supported.

The value of the book today lies not, however, merely in the things that are said, but in the cogent marshaling of facts to back up the arguments and in the clarity of the goal.

The Harvard University Press has performed a real service to all doctors and laymen interested in more than the individual patient. This report should, at the very least, be perused by all such.

F B B

*The Selected Writings of William Clowes* By F. N. L. POYNTER, pp 179, 15s  
Harvey & Blythe Ltd London, W. I. December 3, 1948

William Clowes (1544-1604), an outstanding Elizabethan surgeon, served first in the Earl of Warwick's Army at LeHavre, then later became Surgeon to St Bartholomew's Hospital. Finally, he became Surgeon to the Fleet which defeated

the Spanish Armada A strong-willed man, he represented the best in any medical tradition for he believed in searching for knowledge anywhere—"That is to say, if I find either by reason or experience, anything that may be to the good of the patients, and better increase of my knowledge and skill in the art of surgery, be it either in Galen or Paracelsus, yea, Turk, Jew or any other infidel, I will not refuse it, but be thankful to God for the same "

He was not merely a barber surgeon, for he continually borrowed knowledge from his physician friends and used it whenever he thought it helpful He wrote a book on the treatment of syphilis He told his patients honestly when he could not cure them and whence came the source of their illness "A few years past there repaired unto me a certain man infected with Lues Venerea He earnestly desired me to tell him what his grief might be I answered him secretly, that it came of a naughty, venomous matter 'What do you mean by that?' said he, 'I pray you, speak plainly and openly, for here are none but these two gentlemen, my good friends' It is a spice of the French disease' said I "

Like Paré he knew that when a patient recovered, that it was only that he had dressed him, and that God cured him His forceful language—the first medical writings in the English tongue—is refreshing because it is not cluttered up with medical gibberish and involved rationale He who would fain return to the use of his mother tongue in dealing with scientific subjects will find much delight here

F B B

## BOOKS RECEIVED FOR REVIEW

- A Handbook of Recorded Notations Vocational Nursing* By ALICE L PRICE  
pp 163, \$2 00 C V Mosby Co
- An Introduction to Physics in Nursing* 179 pp \$3 25 The C V Mosby Co, St  
Louis
- Introduction to Organic and Biological Chemistry*, 2nd edition By ARNOW AND  
REITZ 795 pp \$5 75 C V Mosby Co
- Introduction to Psychobiology and Psychiatry*, 2nd edition By ESTHER LORING  
RICHARDS 419 pp \$3 75 C V Mosby Co
- Measurements of the Public Health* By F A E CREW 243 pp 10s net Oliver  
and Boyd, Tweeddale Court, Edinburgh
- Obstetric Analgesia and Anesthesia* By FRANKLIN F SNYDER 401 pp \$6 50  
W B Saunders Co, Philadelphia
- Synopsis of Psychosomatic Diagnosis and Treatment* By FLANDERS DUNBAR  
501 pp \$6 50 C V Mosby Co
- Technic of Medication* By AUSTIN SMITH pp 255 \$4 00 J B Lippincott
- Vocational Nursing for Home, School and Hospital* By ALICE L PRICE pp 344,  
\$4 00 C V Mosby Co

# HUMAN CONJUNCTIVITIS DUE TO NEWCASTLE VIRUS IN THE U S A

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Despite the world wide distribution of Newcastle disease of fowls (1), proved infection of man with this virus has been reported only in Australia (2, 3), and presumed infection of man only in Palestine (6) and, recently, the United States (6). The proved cases of human conjunctivitis due to Newcastle virus here reported establish the pathogenicity of American strains for man, and indicate the likelihood, in the face of broad dissemination of the virus among American flocks (7), of the existence of such infections among our human population.

Three proved cases were reported among Burnet's laboratory workers in Australia. The first (2) developed one day after splashing of infected fluid in the eyes. There occurred a severe, unilateral conjunctivitis and preauricular adenitis, with transitory chills, headache, and malaise. Three days after onset, mild irritation in the other eye was seen. Smears and cultures were negative. The condition cleared in a week. Two later cases (3) presented bilateral and unilateral conjunctivitis, with no systemic symptoms. The author states that in neither of the latter cases was a significant rise in antibody titer demonstrated by serum neutralization of virus hemagglutination. In all cases, Newcastle virus was isolated by inoculation of chick embryos with acute-phase eyewashings.

In Palestine, 17 kitchen workers were afflicted with unilateral conjunctivitis and preauricular adenitis 3 to 4 days after preparing infected poultry (4). A technician who contaminated her eye with Newcastle infected material developed a sub-conjunctival hemorrhage, but no conjunctivitis (5). Neither of these Palestine reports includes virus isolation or serologic studies.

Howitt, et al (6), have reported from Tennessee the discovery of rises in antibody titer to Newcastle virus in paired sera from children who suffered an unexplained febrile illness. This was characterized by



fever, headache, and malaise, being accompanied sometimes by nausea, vomiting and signs of meningeal irritation, and persisting at most for a few days. High antibody titers were seen, too, in isolated serum samples from these patients, whereas none appeared in a control group. Many of the children had contact with chickens. A number of laboratory workers exposed to Newcastle virus showed similar rises after an influenza-like illness. No cases of conjunctivitis were reported.

### CASE REPORTS

*Case 1* L D, handled Newcastle virus contaminated laboratory refuse the day before onset of marked conjunctivitis in the right eye. The right preauricular node became palpable and tender. This condition cleared slowly over 5 days. On the third day, there appeared on the left an acute conjunctivitis and preauricular adenitis which also cleared in 5 days. There was no exposure to contaminated material after onset of the right-sided infection.

Isolation of Newcastle virus from the right eye was successful on the first day and unsuccessful on the second and fourth days of infection. One day after onset of the infection in the left eye, virus was isolated from this eye. Saline eye washings were penicillin-treated and inoculated on the chorio-allantoic membranes of 10 to 12 day chick embryos. Both positive washings killed 3 embryos in 3 days. Allantoic fluids from these eggs agglutinated red cells in a dilution of at least 1/2560. Newcastle-immune chicken serum in a dilution of 1/100 inhibited hemagglutination by 10 agglutinating doses of the virus. Normal chicken serum, beyond a 1/2 dilution, failed to do so.

TABLE 1  
*Serological studies on L D*

DAY AFTER ONSET SERUM TAKEN	SERUM DILUTION NEUTRALIZING VIRUS HEMAGGLUTINATION	SERUM DILUTION AGGLUTINATING VIRUS SENSITIZED RED CELLS	EMBRYO DEATHS WITH $10^{-7}$ VIRUS + SERUM DILUTIONS			
			Undil	1/4	1/16	1/64
0	None with undil serum	1/640	4/4	3/3	4/4	2/4
1	None with undil serum	1/320				
3	None with undil serum	1/640				
6	None with undil serum	1/320				
14	None with undil serum	1/160	0/4	1/4	2/4	2/3
22		1/320				

*Case 2* M F, having harvested infected allantoic fluid the previous day, developed a unilateral, moderately severe follicular conjunctivitis accompanied by

profuse watery discharge and slight preauricular node enlargement The condition cleared in 4 days Scrapings and bacterial cultures were negative

First and second day eye washings killed chick embryos in 2 days Hemagglutination titers of fluids from these eggs rose from 1/20 and 1/40 to 1/5120 and 1/320, respectively on second egg passage The virus was isolated and identified with the same methods as in Case 1 Intramuscular injection of passage I of the first day eye washings into 2 normal chickens caused their deaths in 4 days

TABLE 2  
*Serological studies on M F*

DAY AFTER ONSET SERUM TAKEN	SERUM DILUTION NEUTRALIZING VIRUS HEMAGGLUTINATION	SERUM DILUTION AGGLUTINATING VIRUS SENSITIZED RED CELLS	EMBRYO DEATHS WITH $10^{-7}$ VIRUS + SERUM DILUTIONS			
			Undil	1/4	1/16	1/64
0	1/40	1/20				
2	1/80	1/20				
5	1/160	1/80		0/3	2/3	4/4
142	1/80	1/40		1/3	2/3	4/4

Case 3 J C, worked daily with Newcastle virus in the laboratory She developed moderate, unilateral conjunctival infection and palpebral swellings The preauricular node was palpable and tender Her illness subsided after 6 days

Isolation of virus was attempted unsuccessfully from eye washings taken on the fourth day of the disease

TABLE 3  
*Serological studies on J C*

DAY AFTER ONSET SERUM TAKEN	SERUM DILUTION NEUTRALIZING VIRUS HEMAGGLUTINATION	SERUM DILUTION AGGLUTINATING VIRUS SENSITIZED RED CELLS	EMBRYO DEATHS WITH $10^{-7}$ VIRUS + SERUM DILUTIONS			
			Undil	1/4	1/16	1/64
3	1/40	0	3/3	3/3	4/4	
10	1/80	1/20	1/3	4/4	4/4	
17	1/80	1/10	2/4	4/4	4/4	

#### METHODS

All dilutions were made in buffered saline Washed, human, type "O" red blood cells, in 1 per cent suspension in buffered saline, were used in agglutination tests

Neutralization of hemagglutination was tested by adding 0.5 cc of serum dilutions to 0.5 cc of virus (10 hemagglutinating doses), letting stand at room temperature for 30 minutes, then adding 1 cc of red cell suspension and reading agglutination patterns after 1 hour

Sensitized red blood cells (a washed 1 per cent suspension of cells previously exposed in an incubator to Newcastle virus and by this rendered inagglutinable by the virus) (10) were added in 0.5 cc amounts to 0.5 cc of serum dilutions, and the tubes read after 1 hour at 4°C in Case 1, at 20°C in cases 2 and 3.

Neutralization of virus infectivity was tested by adding equal volumes of serum dilutions and a  $10^{-7}$  virus dilution (about 10 LD<sub>50</sub>'s). One drop of the mixture was inoculated on the chorio-allantoic membrane of 10 to 12 day embryos. The end point was determined by deaths occurring between 1 and 6 days. All serum was frozen at -16 shortly after separation from the clot, and was tested immediately after thawing. Acute and convalescent sera were tested simultaneously.

Strains B and CG 179 of Newcastle virus are used in this laboratory (8).

## DISCUSSION

Clinically, these patients presented an acute, unilateral or bilateral conjunctivitis and preauricular lymphadenitis. The course was brief and without complication, no systemic symptoms were observed. Several other laboratory workers exposed to the virus have developed no illness.

Repeated tests on the sera of these patients reveal a very low antibody response to infection. This and the fact that we had to use a minimal amount of virus (10 L M D) and undiluted sera to demonstrate this rise, suggest that serologic diagnosis of this conjunctivitis may require exceptionally complete studies in order to be significant. Isolation of the virus in the early stage of the disease, when possible, is to be preferred for ease and accuracy.

The slight antibody response may be related to localization of the infection on the conjunctival epithelium. (It is interesting to note that in trachoma and inclusion conjunctivitis, also superficial conjunctival virus infection, there has been found no evidence of specific serological response (9).) On the other hand, Howitt et al (6), present data suggesting that systemic infection with Newcastle virus may stimulate high antibody levels.

Sera from some patients with infectious mononucleosis and other infectious diseases (10, 11) have been found to agglutinate Newcastle virus sensitized red blood cells to a high titer (though not to neutralize Newcastle virus hemagglutination, differentiating them from Newcastle-immune sera). The possibility of a relationship between the causative agents of the diseases has been suggested (10). Sera from

these known human Newcastle infections failed to present similar high titers of sensitized-cell agglutinins

#### SUMMARY

1 The virus of Newcastle disease in chickens in the United States is capable of causing conjunctivitis in man, as demonstrated by the accidental infection of three laboratory workers. Virus was isolated from 2 of the cases.

2 Serologic studies indicate that antibody response is slight in these cases, and that laboratory diagnosis in these superficial infections should rest on isolation of the virus.

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# TREMOR AND CHANGES IN REFLEX STATUS PRODUCED BY DDT IN DECEREBRATE, DECEREBRATE-DECEREBELLATE AND SPINAL ANIMALS<sup>1</sup>

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In the intact mammal DDT (2,2-bis-parachlorophenyl-1,1,1-trichloroethane) produces muscle tremors, dysmetria and hypermetria during walking, exaggerated standing (strong positive supporting reaction) and often an inability to arrest forward progression (1, 8). In both acute and chronic poisoning tonic and clonic convulsions may occur. Since several of these disturbances are ones which characterize the decerebellate animal it has been suggested (1, 5) that in DDT intoxication the cerebellum is selectively affected. An electroencephalographic study of the central effects of DDT in cats and monkeys, carried out by Crescitelli and Gilman (3), showed notable changes in two areas, the region of the motor cortex and certain portions of the cerebellar cortex. In curarized, unanesthetized animals fast waves, completely synchronized in these areas, increased in magnitude and frequency until periodic electrical seizures, corresponding apparently to the tonic-clonic convulsions seen in non-curarized animals, developed. It was concluded that either of the two regions "was firing into the other or both were being simultaneously driven by a group of neurones linked to both." The most definite claim that DDT acts selectively on the cerebellum has been made by Haymaker, Ginzler and Ferguson (5) who found degenerative changes restricted to the cerebellum (chiefly in the dentate and roof nuclei) when they studied sections of the cord and brain from dogs that had been chronically poisoned by relatively large doses of DDT. Other neurohistological studies either failed to demonstrate any abnormalities attributable to the poison (7) or revealed only slight or moderate degenerations in cord (2) or in cord and brain stem (6).

<sup>1</sup> This work was done under contract between the Medical Division of the Chemical Warfare Service and The Johns Hopkins University

On the basis of the clinical symptoms produced by DDT, the electroencephalographic data of Crescitelli and Gilman and their own pathological studies, Haymaker, Ginzler and Ferguson (5) state that "it appears that the cerebellum is the chief portion of the nervous system on which DDT acts" They refer to unpublished work (p 429) in which it was found that after transection of the spinal cord in cats DDT failed to produce tremors in muscles innervated from the isolated cord They also refer to acute experiments in which low mesencephalic truncation of the brain stem failed to interrupt the "neurological symptoms" (presumably the tremor) of DDT poisoning

In some preliminary experiments we noted that in spinal cats typical DDT tremors occurred in muscles innervated from below the level of transection, and a little later we found that in the decerebrate cat complete removal of the cerebellum had little or no effect on the tremors produced in such preparations by DDT These observations suggested that DDT may act quite generally on the central nervous system and led to the work described below in which special attention was devoted to the tremor and to the alterations in reflex status which are induced by DDT

#### METHODS

The animals used in this investigation were cats and dogs A number were surviving (chronic) preparations which were studied some weeks or months after surgical exclusion of one or another part of the central nervous system, or transection of the spinal cord In a larger number of instances we employed acute preparations, animals in which such procedures as decerebration or decerebration combined with spinal section or removal of the cerebellum were carried out shortly before or after administration of DDT Observations of the animal then extended over a period of some hours

In the case of the chronic preparations the operations were carried out with rigorous aseptic precautions and under deep pentobarbital sodium anesthesia (42 mg per Kg body weight, intraperitoneally) In the acute preparations all operative procedures were done under brief but deep ether anesthesia, the rapid recovery from which permitted the central effects of previously administered DDT to show themselves within a short time We repeatedly observed that in decerebrate animals all overt neurological signs of the intoxication quickly disappear with very light etherization and that on withdrawal of ether after deep anesthetization the symptoms reappear with surprising rapidity

DDT was administered in two ways In all experiments on long surviving animals and in one-half of the acute experiments it was administered by stomach

tube in the form of a 10 per cent solution in peanut oil. In a half of the acute experiments it was administered intravenously in an emulsion prepared by the method described by Phillips and Gilman (8). As pointed out by these authors the intravenous method has the advantage that the symptoms develop more rapidly and the response to a given dose is much more regular than when the substance is administered orally (intragastrially). It was found, however, that in decerebrate cats the intravenous administration of the emulsion always produced immediate signs of a central disturbance. Sometimes a bilateral or a unilateral facial paralysis was produced. In those cases in which this was permanent it masked the development of the first signs of intoxication, namely, twitching of the eyelids and face. Also, when the emulsions were used in decerebrate cats the survival times were very much shorter than when the substance was given intragastrially. These untoward effects occurred in spite of great care to use emulsions only immediately after homogenization or re-homogenization and to keep the rate of injection low. Further, it was found that the intravenous injection of the emulsion without DDT, in a volume corresponding to the usual dose per kilogram of body weight, produced not only these same effects but also an extremely fine, very rapid and widely distributed muscular fibrillation which was maintained throughout survivals of several hours. Although this activity was easily distinguishable from the finer components of the tremor evoked by DDT, we not unnaturally concluded that despite certain disadvantages the administration of DDT by stomach tube is the procedure of choice.

Others (8) have noted that the response to the oral (or intragastric) administration of DDT is somewhat capricious and that symptoms appear after considerable delay. In our experience the latency, with doses of from 100 to 400 mg per Kg, varied from 2 to 14 hours, but it did not seem to be related closely to the dosage. On the other hand, doses of 60 mg per Kg, when administered intravenously, caused typical symptoms to appear in from 5 to 35 minutes (average, 19.6 minutes). Accordingly, in the case of acute experiments, when the intravenous route was used, the first operative procedure on the central nervous system was carried out before giving DDT, whereas when the substance was administered by stomach tube the first procedure followed the giving of DDT and was usually performed just after the first signs of an effect appeared.

## EXPERIMENTAL RESULTS

### 1 *Normal Animals*

In order the better to appraise the effects of operative procedures on the development and course of the neuromuscular phenomena produced by DDT a series of normal, wholly intact cats and dogs were given the substance by the intragastric route. In no case did we attempt to produce the chronic state of intoxication described by Bing, McNamara and Hopkins (1). The symptoms which our animals de-

veloped were essentially the same as those described by Phillips and Gilman (8). Typically there appeared, first, rapidly repeated blinking of the eyelids, then fine twitchings of the vibrissae and ears. Next, the activity spread caudally to involve, usually *seriatim*, the muscles of the neck, shoulder girdle, forelegs, hips, hindlegs and trunk. Not infrequently the tail showed tremors and twitches earlier than did hips, hindlegs and lumbar region. In most cases a very fine tremor developed first and persisted, but later a more gross activity, perhaps adequately described as rapidly repeated synchronous "jerkings" of muscle masses, was superimposed on the fine tremor. When the intoxication was sufficient these "jerkings" or "shakes" became tonic-clonic convulsions.

## 2 *Decerebrate Cats (Acute Experiments)*

The effects of DDT were examined successfully in 14 cats which were subjected to removal of all cerebral tissue rostral to transections of the brain stem made at various levels between the middle of the mesencephalon and the upper portions of the pontile region. No differences in symptoms produced by DDT were noted which could be attributed to differences in the level of decerebration.

In 7 of these 14 experiments DDT was given intravenously after decerebration and development of the rigidity and the reflex status characteristic of such preparations. The intervals between decerebration and injection ranged from 22 to 146 minutes (average, 61 minutes). In each case the dose was 60 mg per Kg. In every instance the injection was accompanied by one or more temporary but conspicuous disturbances (opisthotonus, abrupt increase or decrease in rigidity, apnea, gasping, hyperpnea, polypnea, facial paralysis, nystagmus, retching), in two animals a complete facial paralysis lasted throughout the experiment. Following these immediate and, for the most part, temporary changes each of the animals presented in general the features of the typical decerebrate cat. After receiving the injection six of this group remained in good condition for periods which varied from 55 to 129 minutes (average, 96 minutes). One cat died ten minutes after completion of the injection, but before its exitus it developed a typical DDT tremor. Four were subjected to other operations (decerebellation or cord section) after receiving DDT, but there is good evidence



that these procedures had little influence on the survival times (see below) Two succumbed immediately after receiving a second intravenous injection of the emulsion When these survival times are compared with those of the decerebrate cats which received DDT by stomach tube, it becomes evident that intravenous injections of an emulsion containing DDT greatly curtailed the lives of such preparations

Decerebration was carried out in 7 other animals which received DDT, dissolved in peanut oil, intragastrically In one of these the operation was done 4 5 hours before DDT was given In all others of this series decerebration was performed after administration of DDT, the intervals being 3 hours and 18 minutes (3 18), 4 35, 5 00, 1 40, 2 05 and 1 10 Except in the case of the two shortest intervals, nervous symptoms of DDT poisoning appeared before the operation with its attendant etherization was begun Although in 5 of these animals other operative procedures (decerebellation or cord section) were carried out subsequent to decerebration, the animals survived for the following periods of time 24 hours and 15 minutes (24 15), 32 05, 00·39, 23 10, 29 30, 7 30, and 18 55 Four were killed intentionally, one (survival, 7 5 hours) after a severe clonic fit, two died, and one, the animal which survived decerebration only 39 minutes, succumbed in the course of an attempt to remove the cerebellum On the basis of a rather extensive experience in preparing, and studying for the longest possible time, bulbospinal and midbrain cats, it would appear that moderate poisoning with DDT (when it is administered intragastrically) tends to prolong the life of the decerebrate animal<sup>1</sup>

Each of the 14 cats comprising the two groups of decerebrates just described clearly showed the tremor characteristic of intact cats poisoned with DDT As in intact cats it typically began (except when facial paralysis supervened after intravenous administration) in the facial region In several cases, when the effects of the poison were not great, the tremor occurred spontaneously only in the foreparts, especially the face, neck and shoulder girdle When this state of affairs existed, the tremor could be induced in the hindparts reflexly by slight manipulation of the entire animal or by gentle handling of a hindleg or the tail With augmentation and caudal spread of the activity two components, one a fine tremor or fasciculation, the other a more gross

and jerky movement, could be distinguished. Occasionally, especially when the tremor and the twitchings were very vigorous, a convulsive seizure, either clonic or tonic or both, intervened.

It is evident that the most outstanding and most constant feature of DDT intoxication seen in the cat with central nervous system intact, the characteristic tremor, can be evoked by this substance in animals from which all cerebral tissue in front of the hindbrain has been removed. Since the acute bulbospinal cat cannot right itself, stand by itself or walk, these experiments do not answer the question whether the several disorders of stance and locomotion seen in intact poisoned animals are due to interference with forebrain mechanisms or cerebellar-forebrain relationships. These experiments, however, do demonstrate conclusively that DDT exerts profound effects on parts of the neuraxis situated caudad to the mesencephalon and that those effects result in neuromuscular activity which resembles very closely—in fact is indistinguishable from—that evoked by DDT in the animal without any surgical ablation of central nervous tissue.

Evidence was also obtained in these experiments that DDT causes several peculiar changes in the neurological status of the bulbospinal cat. In almost every animal, with the development of tremor and sometimes before any tremor appeared, the forepaws, spontaneously or in response to some slight stimulus of the paw, were strongly flexed and the claws protruded. This occurred when the hindlegs were showing strong extensor rigidity and so produced an attitude which was in strong contrast to that seen in the unpoisoned decerebrate preparation. Another commonly observed response, one rarely if ever evocable in "normal" decerebrate cats, was a bilateral or crossed knee-jerk. On tapping one patellar tendon phasic extension occurred at both knee joints and usually the crossed response was as great as the ipsilateral. While the tonic neck and labyrinthine reflexes seemed unaffected (possibly they were sometimes enhanced), an additional effect of lateral flexion of the head, one we have never been able to evoke in an unpoisoned bulbospinal cat, was almost always encountered—deviation of the tail to the jaw side. This reaction may be called the *ipsilateral head-tail response*. Not infrequently the poisoned decerebrate cats gave the impression of a somewhat generalized increase in reflex excitability, especially of the extensor responses. In a few animals slight

pressure against the toe pads evoked strong extension of the leg. The augmentation of the tremors and twitchings by slight tactile or proprioceptive stimuli was suggestive of a strychnine-like effect. In one animal clapping the hands increased the intensity of the tremor.

### 3 *The Effect of Removal of the Cerebellum in the Acutely Decerebrate Cat*

In 5 cats of the decerebrate series just described ablation of the cerebellum (in each case by gentle suction) was carried out either immediately after decerebration (cats 4 and 18) or after an interval during which symptoms of intoxication were in evidence (cats 2, 3 and 19).

Three of these animals were given DDT intravenously (cats 2, 3 and 4) and were observed under the influence of the poison for relatively short periods of time (48, 43 and 152 minutes, respectively) while in the decerebrate-decerebellate condition. In cat 2 the tremors which had developed in the decerebrate state remained generalized and showed no decrease in vigor, there was some slight increase in the gross jerkings, but this could have been due to the further development of the intoxication. In cat 3 the fine component, the only one present before removal of the cerebellum, largely disappeared and was replaced by a coarse tremor which was maintained until the animal died during a second intravenous injection of the DDT emulsion. Cat 4, subjected to decerebellation immediately after decerebration, developed vigorous generalized tremors and "shakes". At first the grosser component seemed unusually conspicuous, but later there was a marked increment in the fine component.

The two animals of this group, cats 18 and 19, which received DDT by stomach tube were observed in the decerebrate-decerebellate condition for 28.8 and 3.7 hours, respectively. Cat 18 had been given DDT (160 mg per Kg) 100 minutes before completion of the removals, but had failed to exhibit any symptoms over an observation period of more than 11 hours. Another dose, equal to the first, was administered during the twelfth postoperative hour and the first signs of an effect (presumably chiefly due to the first dose) developed about an hour later. Both a fine and a gross tremor appeared in head and tail. For a long time it occurred elsewhere only in response to han-

ding the animal. Toward the end of the experiment spontaneous gross tremors in the axial muscles were combined with fine tremors in the distal portions of the extremities, both were augmented by any slight mechanical disturbance of the animal. On the basis of other experiments it is apparent that no very considerable quantity of the DDT administered reached its sites of action on the nervous system. Cat 19 was decerebrated after the beginning of blepharospasm. During the following three hours it developed very vigorous tremors in all parts of the body and strong gross twitching was superimposed on the fine tremor. Twenty minutes after completion of the decerebellation and withdrawal of ether vigorous twitches were apparent in eyelids, face, neck and tail. Fifteen minutes later almost the entire skeletal musculature showed both the fine tremor and the gross twitching. The twitches became more and more vigorous and finally, 3 hours and 40 minutes after decerebellation (7 5 hours after decerebration), the animal went into a convulsion which began with running movements and developed into violent clonic movements of the entire body attended by piloerection.

The evidence provided by these experiments indicates that the neurological symptoms produced in the decerebrate cat by DDT are not essentially modified by removal of the cerebellum. As indicated above, there may have been some tendency for the gross twitching to be somewhat more marked than in the simple decerebrate preparation, but of this one cannot be sure, the grosser aspects of the DDT tremor usually increase with increasing intoxication and this could have been the cause of the gross twitches noted in cats 2, 3 and 19.

The peculiar changes in reflex status produced in decerebrate cats by DDT and noted above were also seen in the poisoned decerebrate-decerebellate animals.

The development of a convulsive episode in cat 19, which was quite like those seen in intact cats after large doses of DDT, is of special interest, for it suggests that in the intact animal neither cerebrum nor cerebellum is essential for this feature of DDT intoxication. It is very possible indeed that had other cats of this series received larger doses of DDT they too would have developed convulsions, several had fits of running movements.

#### 4 *An Observation on a Chronically Decerebellate Dog*

That the presence of the cerebellum is not essential for the occurrence of a DDT convulsion was further demonstrated by an experiment carried out on a dog twenty-three months after complete removal of his cerebellum. During this long postoperative period the animal had been studied intensively in the course of another investigation. He had remained in excellent health and exhibited all the characteristics shown by dogs after uncomplicated extirpation of the cerebellum. At 4 15 p m the animal was given DDT dissolved in peanut oil in a dose amounting to 200 mg per Kg of body weight. At 6 30 no symptoms had appeared and the animal was left unobserved. At 8 30 (4 25 hours after DDT) the dog was found panting heavily and noisily and shaking so violently that the loud rattling of the cage attracted attention in a far part of the building. On removing the animal from its cage it at once fell onto its side, rolled over, urinated, and went into a tonic convulsion with all limbs flexed and back deeply concave. Death occurred in two or three minutes. It is unfortunate that the observation was so brief, but it was clear enough that the animal had developed a violent attack of DDT "shakes" which culminated in a tonic convulsion.

#### 5 *Spinal Animals*

*A Acute Experiments on Cats* In 4 cats of the decerebrate series decerebration was followed by transection of the spinal cord at a thoracic level. After this procedure the muscles of the pelvic girdle, hindlegs, and tail were innervated from an isolated cord, while the innervation of the foreparts remained "decerebrate". A comparison of the motor activities of the two portions of the body permitted some judgment of the susceptibility of spinal mechanisms, freed from all supraspinal influences, to the action of DDT.

Cat 4 was given DDT intravenously (60 mg per Kg) after decerebration and decerebellation. Seventy-two minutes later, when very vigorous generalized tremors had developed, the cord was transected between the 11th and 12th thoracic segments. Five minutes after withdrawal of the ether a fine tremor was present in hindlegs and tail. Seventeen minutes later the tremor was as marked in hindlegs as in

forelegs Before the animal died during a second intravenous injection of DDT there was a marked increase in the vigor of the tremors and twitches of parts innervated from above and from below the transection Throughout the occurrence of tremor in the parts innervated by the isolated cord the knee-jerks were bilateral (crossed) and the hindlegs assumed an attitude of flexion and markedly resisted passive extension In cat 5 the cord was cut at the same thoracic level twenty-five minutes after decerebration and thirty-three minutes before DDT was given intravenously (60 mg per Kg) Fifteen minutes after the injection small tremors began simultaneously in all four legs During the rest of the period of survival (the cat died 95 minutes after the injection) both ends of the cat showed typical tremors and in each part they became more vigorous when that part was manipulated<sup>2</sup>

In two cats (Nos 12 and 15), which had received DDT by the intragastric route and were showing good tremors and twitches throughout the body after decerebration, the spinal cord was transected at a mid- or high thoracic level (in cat 12 at T6, in cat 15 at T3) Of these cat 12 showed the greater effect, doubtless because it was given a larger amount of DDT 200 mg per Kg initially and 400 mg per Kg just after section of the cord During the first six hours following the transection there was a slow but steady increase in the spontaneous tremors and twitches of all muscles innervated from parts of the neuraxis lying above the section, but tremors appeared in the hindparts only reflexly A very slight passive movement of one hindlimb sufficed to induce kicking movements of both hindlegs and, in all parts innervated from below the section, vigorous tremors which were somewhat more gross than those occurring spontaneously in the foreparts During this same time there developed other signs of an altered reflex status of the isolated cord The knee-jerks were bilateral and the

<sup>2</sup> It should be stated here that in cat 5, as in several other preparations which were showing typical DDT tremors in hindlegs and tail, the femoral and sciatic nerves were cut on one side and the caudal segments of the spinal cord removed These procedures put an end to all tremors in the tail and in the limb muscles whose innervation was thus interrupted Such observations, however, do not prove that in the mammal DDT has little or no peripheral action Indeed we have recently learned of evidence that the substance has a veratrinic action on nerve and on curarized muscle (4)

hindlegs, kept in a semi-flexed position, strongly resisted passive extension. These alterations in cord activity remained throughout the survival of the preparation. A tremor appeared spontaneously in the tail 6.25 hours after cord section. At this time the head and forelegs had long been showing gross jerks superimposed on a fine tremor. Within the next three hours the hindlegs began to show spontaneous tremorous activity, but while this was qualitatively the same as that going on in the foreparts it was not as vigorous. This picture was maintained for approximately 7.5 hours. During this time the isolated cord developed an extraordinary reflex excitability which is never seen in the ordinary acute or semi-chronic spinal preparation. The hindlegs showed ipsilateral and crossed knee-jerks, a good mark-time reflex, the lengthening reaction and its crossed component (Philippson's reflex) and the tonic flexion mentioned above. The flexion reflex of each hindleg acquired an amazingly low threshold, a light touch applied between the toes evoked strong withdrawal of the foot. During the last five hours of this cat's survival there was a gradual regression of the changes produced by the DDT, the spontaneous tremor weakened in the foreparts and disappeared in the hindparts where, however, it could still be induced by manipulation of tail or of a hindleg. Finally, tremors could be induced only reflexly in the foreparts.

In cat 15 the same procedures were carried out as in cat 12, and in the same sequence. It received an initial dose of 160 mg per Kg and a second dose of the same amount just before the cord was transected. Following the transection this animal displayed very much the same activity as did cat 12. In hindlegs and tail the tremors occurred spontaneously, but they never became as vigorous as in the foreparts.

*B Chronic Spinal Preparations* Although the results described above leave no doubt that DDT can act on the isolated spinal cord to produce tremor and changes in reflex status and threshold, it seemed desirable to test the effects of the substance in animals which had long survived a transection of the cord. This was done in two cats and two dogs.

Cat A3 was given DDT (160 mg per Kg) by stomach tube 69 days after transection of the cord at the second thoracic segment. After 2.3 hours a slight tremor appeared in the foreparts when the

animal was moved. It was not until 6 hours had elapsed that blepharospasm and a tremor in the forelegs occurred spontaneously. At this time the knee-jerks became crossed (bilateral), probably the first indication of an effect upon the isolated cord. An hour later a rather gross tremor was present in the foreparts and tremors appeared in hindlegs and tail when one or the other was manipulated. For another hour the tremors remained spontaneous in the head and forelegs, only reflexly elicitable in tail and hindlegs.

Cat A5 showed a more pronounced effect of the same amount of DDT (160 mg per Kg). The experiment was done 37 days after transection of the cord between the third and fourth thoracic segments. Definitely characteristic DDT tremors appeared spontaneously in hindparts and foreparts 2.5 hours after the substance was given by stomach tube. In the hindlegs the tremors were less continuous and not as fine as in the forelegs. The tail was now showing marked gross twitches. During the next 2.5 hours the activity became greater in all parts, but the same difference was maintained between the normally innervated and the spinally innervated regions. Early in the sixth hour the animal had a convulsion and died. Just before this episode the hindlegs and tail exhibited strong gross tremors. They did not take part in the convulsive activity.

Dog A received DDT (150 mg per Kg, intragastrically) at 3.40 p.m. on the fifteenth day after transection of the cord between T10 and T11. A slight tremor appeared in the foreparts at 5.50 and during the next hour gradually increased in vigor. Blepharospasm was present, but not continuous and not very strong. Aside from an augmentation of the knee-jerks, which became "decerebrate" in type, the hindparts showed no evidence of any effect until 7.00 when the hindlegs began to show a definite tremor. At 8.00 the dog became excited, vocalized, was unable to right the shoulder girdle and executed some running movements with the forelegs. Then the part of the body above the transection developed a tonic spasm with forelegs rigidly extended, head thrust forward, jaws open and mouth frothing. During this seizure vigorous tremors occurred in face and forelegs. While the hindparts did not take part in the fit, they showed tremors which were entirely unrelated in phase or intensity to the activities which occurred in the foreparts. At 8.15 the rectal temperature was 41.5° C.



and enough pentobarbital sodium was given to produce relaxation. By 8.30 the animal was entirely quiet and relaxed except for a fine tremor in the foreparts. When she had recovered from the effects of the barbiturate twelve hours later no trace of tremor was observable in any part of the body.

In a second dog (No. SD1) the cord was transected at a mid-thoracic level and the effects of DDT were tested on five occasions between the 13th and the 27th postoperative days. The initial dose varied from 150 to 250 mg per Kg and in one test was twice repeated. In two of these five experiments, with single doses of 200 mg per Kg, the effects produced were so slight that they need not be considered here. On the first test, begun on the 13th day, 150 mg per Kg were given. A tremor of high frequency and small amplitude appeared during the seventh hour both in the normally innervated foreparts and in the spinally innervated hindparts. A little later some gross twitching was added to the fine tremor in hindlegs and tail. With this the hindlegs showed increased extension. The tremor disappeared after the twenty-fourth hour. Three days later, when 200 mg per Kg were given, tremors appeared in all parts of the body after three hours, became maximal after seven hours and then decreased until, during the twenty-third hour, they were present only in the foreparts. In the muscles innervated from the isolated cord the tremors were never as rapid, vigorous or continuous as those occurring concurrently in the foreparts. A second dose of 200 mg per Kg was given 22.3 hours after the first, and within two hours the tremor reappeared in the hindparts. It was as strong and as fine in character, but not as continuous, as that in the foreparts and it continued for more than five hours. At the end of the thirteenth hour tremor was absent from the foreparts, but a very slight one could be made out in the hindlegs. This unusual situation still prevailed after 26.5 hours, a discontinuous fine tremor was still evident in the hindlegs, but no tremor of any kind was observable in the foreparts. At this time (it was the 19th postoperative day) a third dose of 200 mg per Kg was administered and gave rise to a very marked continuous tremor in head, shoulders and forelegs and a somewhat less vigorous and slightly intermittent one in the hindlegs and tail, both of which effects persisted for many hours.

Finally, on the 26th day, 250 mg per Kg led to a continuous very strong tremor in all parts of the body musculature. Gradually, over a period of 8.5 hours, this activity diminished until only the forelegs showed a slight tremor, the hindparts having become quiescent some time before.

In two of the tests carried out on dog SD1 oscillographic records of the electrical activities of foreleg and hindleg muscles were taken from small wire electrodes, insulated down to their tips, which were thrust into a flexor or extensor muscle. These records indicated that, except for some intermittency and some deficiency in frequency, the tremorous contractions of groups of fibers in hindleg muscles were qualitatively the same as those in muscles of the forelegs. On comparison with records taken when the animal was not under the influence of DDT, they also gave evidence of an action of the poison on the reflex status of the isolated cord, an action which was also evident on simple visual observation of the reflexes. This consisted in signs of increased extensor tonus, augmentation of knee-jerks and the reflex of crossed extension with an enormous prolongation of after-discharge. Even the knee-jerks showed after-discharge. In the electrical records the after-discharge of the crossed-extension reflex, evoked by pinching the foot of the opposite hindleg, was found to last as long as 1.5 minutes. In the case of the knee-jerk the electrical activity of the responding muscles showed that muscle units fired repetitively for many seconds after the initial discharge. On visual observation the leg showed corresponding clonic extensions. Thus it appears that DDT acts on the isolated cord of the dog not only to produce tremors, but also to bring about a reflex status which resembles that of the decerebrate condition.

#### SUMMARY AND CONCLUSIONS

1 In acutely decerebrate cats DDT produces a tremor which is indistinguishable from that induced in normal animals by the poison. It also causes signs of an altered central state or "set of the center," which is indicated by a tendency of forepaws to be flexed with claws protruded, crossed (bilateral) knee-jerks, appearance of the "ipsilateral head-tail response," and some increase in reflex excitability, especially of extensor responses.

2 The effects produced by DDT in the decerebrate cat are not essentially modified by removal of the cerebellum. In the decerebrate-decerebellate cat DDT may evoke a tonic-clonic convulsion. A seizure of this type was seen in a dog which was given DDT twenty-three months after total uncomplicated ablation of the cerebellum.

3 While these experiments demonstrate that forebrain, mesencephalon and cerebellum are in no way necessary for the development of the tremor characteristic of the intact animal poisoned by DDT, they do not permit any conclusion as to the site or sites of the action of this substance which results in abnormalities of stance and locomotion in the animal with central nervous system intact.

4 DDT produces tremor in muscles innervated from segments of the spinal cord which have been deprived of all neural connections with the brain. This fact has been shown in animals with cord transected at a thoracic level and studied either in the chronic spinal state or in the acute decerebrate condition. When such animals are given DDT the tremor usually appears first in the foreparts. When the intoxication is light, as judged by the behavior of the foreparts, the tremor may appear only reflexly in the hindparts in response to a mechanical disturbance of that portion of the body. With deeper levels of poisoning, tremor occurs spontaneously in the hindparts, but may not attain the vigor of the activity present in muscles innervated from above the transection. Sometimes, but by no means invariably, the tremor in the muscles innervated from the isolated cord is not as fine as that in the foreparts, the coarse component being more prominent.

5 DDT acts to alter the reflex activities of the isolated lumbosacral cord. In the case of the cat there is augmentation of flexor tone, lowering of threshold of the flexion reflex, and irradiation of impulses from proprioceptors of the knee extensors to give a crossed knee-jerk. In the spinal dog the reflexes involving hindleg extensor muscles become more active and show very long after-discharges.

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# A CONSTANT DEGREE OF ANOXEMIA OBTAINED BY THE ADMINISTRATION OF A GAS OF VARIABLE OXYGEN CONCENTRATION<sup>1</sup>

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## INTRODUCTION

In a previous report a method of producing a constant degree of anoxemia was described (1). It consisted of altering the oxygen concentration of the inspired gas mixture as necessary to give the desired degree of arterial oxygen saturation, as measured by the oximeter. The inspired gases were administered by means of an anesthesia machine containing tanks of pure oxygen and pure nitrogen.

It was noted at that time that frequent changes in the flow rate of oxygen and nitrogen were necessary to maintain the arterial oxygen saturation at 85, 80 or 75%. It is the purpose of this communication to present data obtained by gasometric analysis of the inspired gas during such controlled anoxemia tests and to describe modifications of and further experiences with the controlled anoxemia technique, with especial reference to the comparison of this procedure with the administration of a low oxygen gas of constant concentration.

## METHOD

All subjects were young adults on whom a previous physical examination and electrocardiogram were found to be normal. The method of inducing and maintaining the desired level of anoxemia was essentially the same as that described in the earlier report, save that a mouth-piece and nose clip replaced the O E M mask. The anesthesia machine and tubing were tested to detect any possible leakage from the outside air. In the experiments in which a gas with constant oxygen percentage (10.7% O<sub>2</sub>) was used, the tank containing this gas replaced the nitrogen in the anesthesia machine. The subject rested for ap-

<sup>1</sup> Supported by a grant from the United States Public Health Service, Federal Security Agency.

proximately 30 minutes until the blood pressure and pulse reached resting levels (available from previous studies done on the same subject). During this period, the oximeter earpiece was attached to the subject's ear and "ear thickness" readings were taken to determine when vasodilatation was complete. Figure 1 illustrates the apparatus described above in use during an actual experiment.

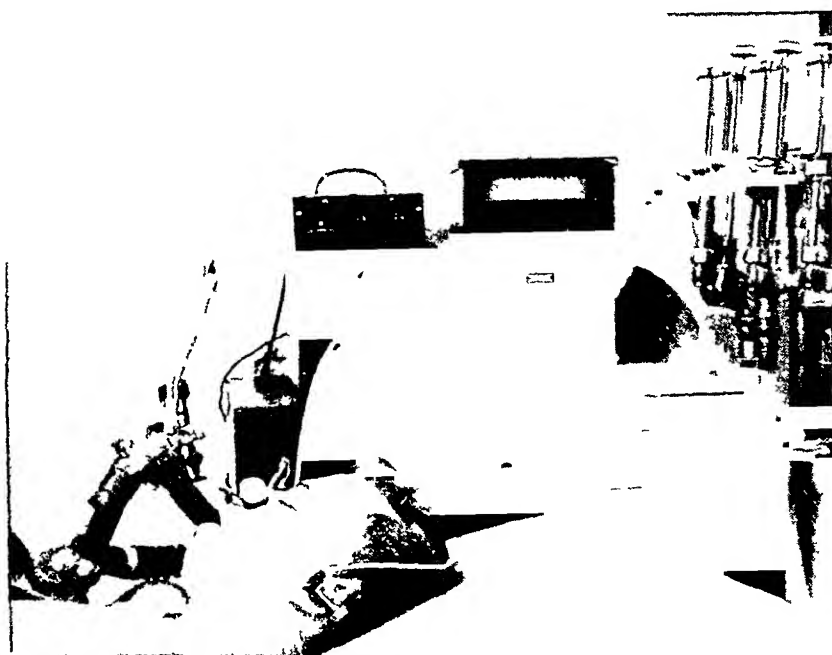


FIG 1

Samples of the inspired gas were taken only after the arterial oxygen saturation had been constant, at the desired percentage, for at least  $1\frac{1}{2}$  minutes. The samples were taken into Bailey bottles (2) from a tube connecting the inflow tube of the gas machine to the breathing bag. Each sample was then passed through a drying column of calcium sulphate to a Pauling Oxygen Analyzer, Model C, for direct measurement of the oxygen tension. The  $pO_2$  values thus obtained were converted to oxygen percentage. The Pauling apparatus was checked frequently against a Scholander analyser (3) and found to give readings consistently 1-3 mm higher than obtained with the Scholander.

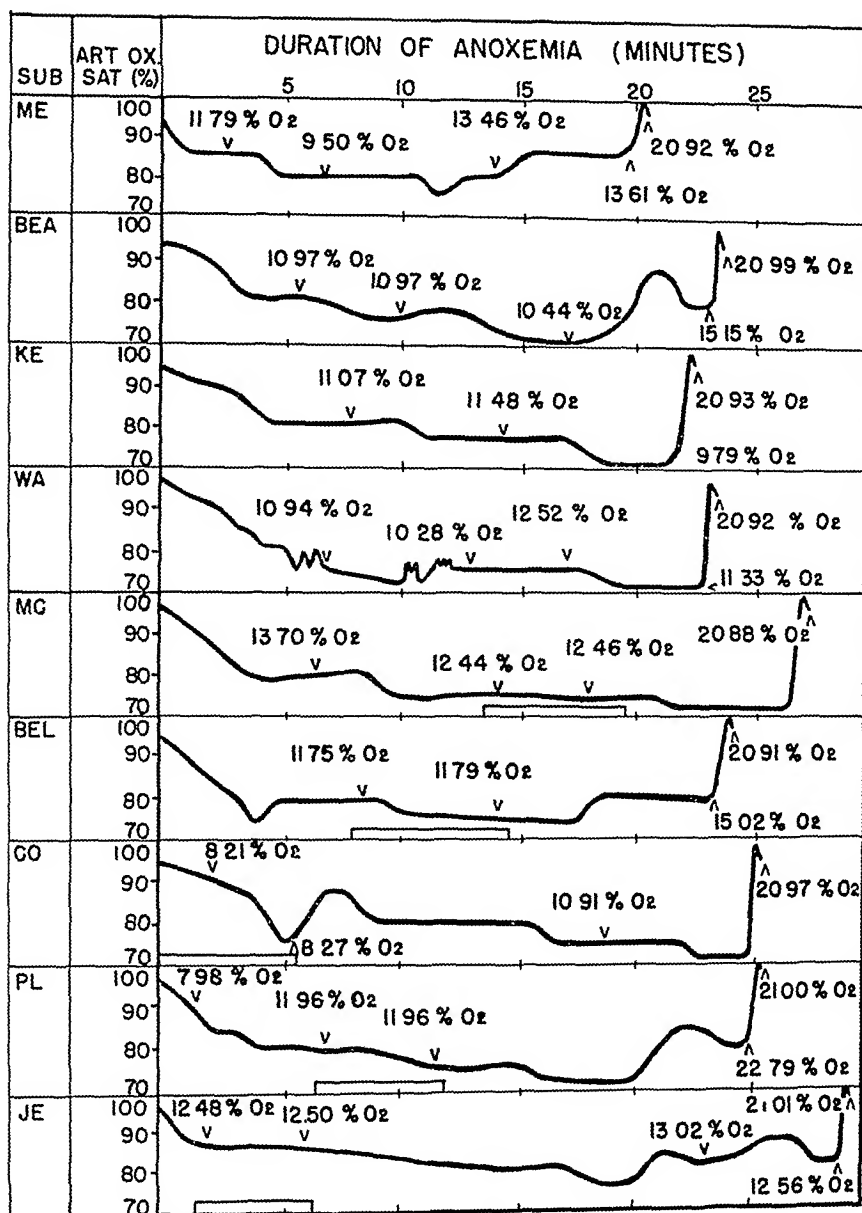


FIG 2 GRAPHIC ILLUSTRATION OF THE RELATIONSHIP OF THE ARTERIAL OXYGEN SATURATION AND OXYGEN CONCENTRATION OF THE INSPIRED GAS

— indicates period during which oxygen and nitrogen flow rates were not changed    v indicates point at which gas sample was taken

TABLE 1

*The Oxygen Concentration of the Inspired Gas with Progressively Decreasing Arterial Oxygen Saturation*

SUBJECT	ARTERIAL OXYGEN SATURATION	OXYGEN CONCENTRATION OF INSPIRED GAS
	%	%
Me	85	11 79
	80	9 50
Bea	80	10 97
	75	10 97
	70	10 44
Ke	80	11 07
	75	11 48
	70	9 79
Wa	75-80	10 94
	75	10 28
	70	11 33
Mc	80	13 70
	75	12 44
Bel	80	11 75
	75	11 79
Co	91	8 21
	75	8 27
Pl	90	7 98
	80	11 96
	75	11 96
Je	87	12 50
	80	13 20

## RESULTS

*The Variability of the Oxygen Concentration of the Inspired Gas with Constant Arterial Oxygen Saturation*

A total of 41 oxygen tension analyses was done on 9 subjects. The relationship of the oxygen percentage of the inspired gas to the arterial saturation is graphically presented in Figure 2.

In Table 1 the values of the oxygen concentration obtained at



*progressively decreasing* arterial oxygen saturations are recorded. The poor correlation of the oxygen concentration and the arterial saturation is striking even in this small group.

In Table 2 analyses of the oxygen percentage of gas samples obtained at the *same* arterial saturation in the *same* individual are compared. In these six instances, the subjects, after being taken to progressively lower levels of arterial saturation were made gradually *less* anoxic towards the end of the experiment. In this manner two gas analyses could be made at one particular saturation. The widely varying value of the oxygen percentage, even when the arterial saturation is the *same*, is again striking. It is of interest to note that, in five of the

TABLE 2

*The Oxygen Concentration of the Inspired Gas at the Same Arterial Oxygen Saturation*  
#1 Gas sample was taken *before* #2 at the particular arterial saturation

SUBJECT	ARTERIAL OXYGEN SATURATION	OXYGEN CONCENTRATION OF INSPIRED GAS	
		#1	#2
	%	%	%
Mc	80	9.50	13.46
Bea	80	10.97	15.15
Bel	80	11.75	15.02
Co	75	8.27	10.91
Pl	80	11.96	22.79
Je	80	13.02	12.56

six instances, the oxygen concentration obtained *after* the subject was brought up from the lower level was from 2.6 to 10.8% higher than the concentration necessary to produce the same degree of anoxemia earlier in the experiment.

In evaluating the gas analysis data, it is appropriate to consider to what extent these data may have been affected by experimental error in gas analysis and in oximetry. To establish the limits of accuracy of the gas analysis the following procedure was carried out with five subjects (Mc, Bel, Co, Pl, and Je in Figure 2). While the oxygen and nitrogen flow rates remained constant, two samples of gas were taken several minutes apart for comparison. The difference in oxygen percentage between the five sets of samples was .02, .04, .06, .00 and 0.02%. It, therefore, was felt that errors from this source were insig-

nificant Error from oximeter "drift" was also relatively small, never exceeding  $2\frac{1}{2}$  percentage units

*Stability of the Arterial Oxygen Saturation with Oximeter  
Controlled Anoxemia*

79 anoxemia tests with oximeter control were done on 59 subjects. In the beginning of this series the arterial saturation was lowered to 85% or 80% and kept at this level for the entire anoxemic period (approximately 20 minutes) with the intention of studying the subjects at lower levels on subsequent occasions. It was later decided, however, to induce anoxemia in a "stepwise" manner, i e, decreasing the saturation to successively lower levels during the same test. When this procedure was used, the subject was usually kept at each level for approximately 5 minutes. The number of tests are given below according to the saturation to which the subject was initially taken, and at which he was subsequently maintained.

NO OF TESTS	INITIALLY TAKEN TO	MAINTAINED AT
	%	%
4	85	85
19	80	80
31	80	80 and 75
16	80	80, 75 and 70
7	75	75
2	75	75 and 70

In 49 of these 79 tests the subject could be brought to the desired level of saturation within a half minute of the time desired, and be kept within 1% of this level throughout the entire period of anoxemia. In 14 tests the subject usually was within 1-2% of the desired saturation or occasionally deviated by a few percent for a minute. In 10 tests, the saturation fluctuated about 2% from the desired arterial oxygen saturation, swinging regularly around the desired percentage of arterial saturation. With 6 subjects, no stable level of arterial saturation could be induced or maintained on the first test. Four of these 6 subjects had repeat tests, three of which were perfectly stable and the fourth unstable.

The induction and maintenance of a constant, steady arterial oxygen saturation, regardless of level, depended chiefly on the *gradual* induction of anoxemia, 5 minutes were necessary to bring a subject from the normal saturation point of approximately 97% (on room air) down to 80%. It was found that at this rate of induction, the subject could be brought to 80% and kept at a constant level without "over-shooting". The changes from 80% to 75%, and from 75% to 70% were carried out in  $1\frac{1}{2}$  minutes in order to prevent similar over-shooting and general instability of the degree of saturation.

In general, a particular individual was just as stable at one level as at another. There was also little difference in stability of the test between the first 10 and the second 10 minutes of the test.

In summary, practically all subjects could be induced to levels of 85%, 80%, 75% (and some 70%) saturation and maintained at these levels for the desired length of time.

#### *Variability of the Arterial Oxygen Saturation with Inhalation of Low Oxygen of Constant Concentration*

A demonstrative group of experiments illustrating the nature of the variability of the arterial oxygen saturation with inhalation of a constant low  $O_2$  gas is presented in Figure 3. Here a few relaxed subjects, entirely familiar with the procedure, inhaled the 10.7%  $O_2$  for approximately twenty minutes under ideal laboratory conditions. Only one subject (Figure 3a) had two "smooth" tests in both of which his arterial oxygen saturation was approximately the same. Another subject (Figure 3b) had a higher and more unstable saturation in a repeat test showing considerable variation with each respiration. In Figure 3c the arterial saturation of 4 subjects is compared showing a variation not only in the *depth* of saturation attained at the end of the twenty minute period, but also a variation in the *rate* with which the arterial saturation was lowered.

In contrast to this, oximeter control of anoxemia was used on three of these same subjects on other occasions. In every instance it was possible to induce the subject to the desired level of arterial saturation (75%) in a standard length of time (5 minutes) and to keep the saturation within 1-2% of this level throughout the remainder of the test.

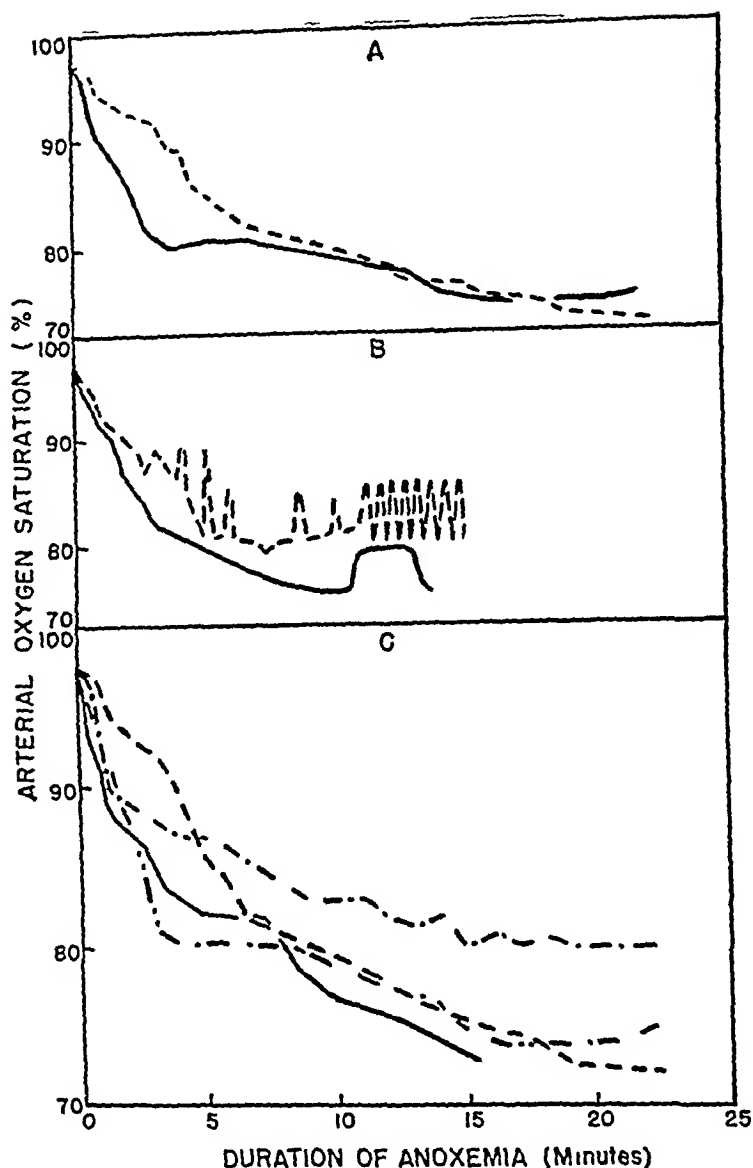


FIG 3 DIFFERENCES IN THE RESPONSE OF THE ARTERIAL OXYGEN SATURATION WITH INHALATION OF THE SAME LOW OXYGEN GAS (10.7%  $O_2$ )

In A, one subject with two relatively similar tests. In B, another subject with two strikingly different tests. In C, the variability of the rate and degree of arterial oxygen "desaturation" with inhalation of 10.7%  $O_2$  by four different subjects.

## DISCUSSION

*The Relationship of the Oxygen Concentration of the Inspired Gas to the Degree of Anoxemia*

The data presented in Figure 1, and Tables 1 and 2, are in complete agreement with Malmstrom's statement that the oxygen concentration of the inspired air is a "poor index" of the resulting degree of anoxemia (4). This disparity between the oxygen percentage of the inhaled gas and the resulting degree of anoxemia is in agreement with the observations made by Hohwu-Christensen and Krogh (1935). As quoted by Malmstrom (4), they "examined 50 healthy flyers by letting them rebreathe in a gas tank of 200 liters while simultaneously absorbing carbon dioxide. The test continued till the subjects became so strained that they were not capable of performing qualified work. When the test had to be interrupted, the alveolar oxygen tension was found to be approximately the same (about 30mm Hg) in all subjects. The oxygen percentage in the inspired gas varied a great deal at that moment between 5.93 and 9.97 volume percent."

From the data given in Table 2 another point of physiologic interest can be made—that the *longer* one is anoxemic, the *higher* must the oxygen concentration of the inhaled gas be to maintain a given level of arterial oxygen saturation.

The chief inference to be drawn from these data is that control of the arterial oxygen saturation in any anoxemia study requires that the oxygen percentage of the inspired gas be altered almost constantly.

*The Advantages of Oximeter Control of Arterial Oxygen Saturation in Anoxemia Studies*

1 *Control of the Degree of Anoxemia* Under "Results" it has been shown that practically all of the 59 unselected healthy subjects could be induced to and maintained at the desired level of arterial oxygen saturation. This is the chief advantage of the controlled anoxemia method and can best be appreciated when one considers the variability of the arterial oxygen saturation resulting from the inhalation of a gas of fixed low oxygen concentration. This subject has been extensively reviewed by Malmstrom (4). The arterial oxygen saturation with inhalation of 8%, 9%, 10% and 12% oxygen, as recorded by various workers, is shown in Table 3. The variability of the satura-

tion is striking throughout, regardless of method or type of subject used

The importance of being able to control the degree of anoxemia in any anoxemia test, e.g., of cardiac function, has also been mentioned and stressed by Barach and Steiner (5f), Mannheimer (6a), Houston (6b), Malmström (4), Landgren (5e), Björck (6c) and Dupps and Comroe (5a). Work done on animals and human beings has emphasized the need for a method of controlling the level of arterial oxygen saturation in studying the many physiological effects of anoxemia. Wiggers (7a) has reported that when the arterial oxygen saturation is lowered from 55% to 35%, a "cardiovascular crisis" supervenes. Hilton and Eichholtz (7b), measuring the coronary blood flow of dogs directly, showed that there was a direct relationship between the arterial oxygen saturation and the rate of coronary blood flow. In human beings, increase in heart rate (5a), activity of the carotid body (8a), visual acuity (8b), and integration of the central nervous system (8c) have been found to have a direct relationship to the degree of arterial oxygen saturation.

2 *Control of the Rate of Induction of Anoxemia* The work of Malmström clearly shows that in a group of normal subjects inhaling the same low oxygen gas, the rate of arterial oxygen "desaturation", as well as the degree, varies considerably. Administering 9%  $O_2$  to 19 normal subjects, he found the average arterial saturation to be 10.5% lower after 15 minutes than at the end of 5 minutes. The rate of desaturation was quite irregular with the difference between 15 minute and 5 minute values ranging from -22% to +8%. These variations in rate and degree of desaturation are demonstrated even in our own small group of subjects breathing 10.7%  $O_2$  (Figure 3).

The physiologic significance of being able to standardize the rate of induction of anoxemia has been clearly demonstrated in animals (9). Those animals made rapidly anoxic showed a qualitatively different cardiovascular response from those made gradually anoxic. Also, rapid induction often produced convulsions. The uncontrolled rate of induction with the administration of a gas of fixed oxygen concentration may well account for the occasional serious nervous symptoms and cardiovascular collapse reported with the 10%  $O_2$  test of cardiac function (10, 6c).

TABLE 3

*The Variability of the Arterial Oxygen Saturation with Inhalation of 8%, 9%, 10% and 12% Oxygen*

% O <sub>2</sub>	AUTHOR	METHOD OF INDUCING ANOXEMIA	METHOD OF MEASURING ARTERIAL OXYGEN SATURATION	TYPE OF SUBJECT	NO OF SUBJ	ARTERIAL OXYGEN SATURATION			
						Mean (%)	Std dev	Std err mean	Range (%)
8	Dripps and Comroe (5a)*	Tank (of low O <sub>2</sub> gas)	Oximeter	Normal	11	72.8	4.9	1.47	64-79
	Wood and Geraci (5b)	Tank	Blood (Gas Analysis)	Normal	10	62.2			53-69
	Rahn and Otis (5c)	(High Altitude) Chamber (22,000 ft)	Oximeter	Normal	8	69.0			61-79
9	Henson et al (5d)	Chamber (20,000 ft)	Oximeter	Normal	9	70.8	5.83	2.06	
	Malmstrom <sup>8</sup> (4)	Tank	Blood	Normal	29	58.8	9.6	1.78	44-82
	Rahn and Otis	Chamber (20,000 ft)	Oximeter	Normal	8	74.0			
10	Dripps and Comroe*	Tank	Oximeter	Normal	34	73.8	6.6	1.15	54-85
	Henson et al	Chamber (18,000 ft)	Oximeter	Normal	13	71.2	6.54	1.89	
	Lindgren (5e)	Tank	Oximeter	Normal	11	71.0			
	Wood and Geraci	Tank	Blood	Normal	9	72.4			66-77
	Rahn and Otis	Chamber (18,000 ft)	Oximeter	Normal	8	75.0			68-78
	Barach and Steiner (5f)	Tank	Blood	Normal	8	65.9			52-78

\* Statistical values calculated from the authors' data

TABLE 3—*Continued*

C. O.	AUTHOR	METHOD OF INDUCING ANOXEMIA	METHOD OF MEASURING ARTERIAL OXYGEN SATURATION	TYPE OF SUBJECT	NO OF SUBJ	ARTERIAL OXYGEN SATURATION			
						Mean (%)	Std. dev.	Std. err. mean	Range (%)
12	Dripps and Comroe*	Tank	Oximeter	Normal	32	80.2	5.6	99	68-88
	Henson et al	Chamber (14,000 ft)	Oximeter	Normal	17	79.2	4.05	1.01	
	Wood and Geraci	Tank	Blood	Normal	9	79.7			70.4-95.0
10	Barach and Steiner**	Tank	Blood	Cardiac	28	67.6			55-89
	Lindgren	Tank	Oximeter	Cardiac	13	69			
12	Levy et al (10)	Tank	Blood	Cardiac	17	70.9			50.1-84.6

\*\* One cardiac patient, whose blood arterial oxygen saturation fell to 34% with inhalation of 10% O<sub>2</sub>, was excluded from calculations as the control value was only 76% on room air.

The use of a variable low oxygen gas to induce anoxemia enables us to control the rate with which a subject is taken to the desired level of arterial oxygen saturation. This should, therefore, help standardize the cardiovascular response to anoxemia and avoid some of the nervous symptoms.

*3 Knowledge of Arterial Oxygen Saturation During the Test* Another definite advantage of any procedure in which the oximeter is used is that the arterial oxygen saturation is known instantly at all times. The importance of avoiding saturations as low as 50% in normal subjects and cardiac patients inhaling fixed low O<sub>2</sub> gases has been mentioned previously (1). Workers using 8%, 10% and even 12% O<sub>2</sub> have reported that in many instances tests had to be terminated before the end of the usual 10 or 20 minute period because of the occurrence of symptoms which could well have been due to an unexpectedly low arterial saturation. With the oximeter one could readily terminate the test if the saturation for any reason took a precipitous drop.

The oximeter also allows us to measure the arterial saturation of a



normal subject even while he is still breathing room air by comparing this saturation with that obtained during the inhalation of pure oxygen (11) This procedure may prevent the inadvertent administration of a low oxygen gas to a subject whose arterial blood is abnormally unsaturated while breathing room air (5f)

#### SUMMARY

Further experiences with the use of a low oxygen gas of variable concentration to induce a constant degree of anoxemia are presented The oxygen concentration of the inspired gas was analyzed with a Pauling oxygen analyzer, and it was established that the concentration had to be almost continually altered to induce and maintain the desired arterial oxygen saturation in a small group of normal subjects

The technique of administering this variable oxygen mixture was used in 79 anoxemia tests in which the subject was lowered to levels of 85, 80, 75 and 70% arterial saturation Practically all subjects could be induced to these levels at a standardized rate and maintained at the desired level for the remainder of the test

The advantages of the "controlled anoxemia" technique over the administration of a gas of *fixed* low oxygen concentration are (1) it controls the degree of anoxemia (2) it controls the rate of induction of anoxemia and (3) the arterial saturation is known instantly throughout the test The physiologic importance of oximeter control of anoxemia is discussed

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# THE TREATMENT OF ACUTE BRUCELLOSIS WITH AUREOMYCIN<sup>1 2</sup>

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Despite the advances in chemotherapy of bacterial disease since the introduction of the sulfonamides, penicillin and streptomycin, the treatment of brucella infections has been a therapeutic problem (1) In a preliminary report describing the early clinical trial of aureomycin, a patient ill with brucellosis, who had been successfully treated, was reported (2) Subsequent observation of this patient, together with further experiences with this antibiotic in the treatment of four additional patients, are presented at this time to supplement the earlier report

## IN VITRO STUDIES

The sensitivity to aureomycin *in vitro* of eleven recently isolated strains of *Brucella suis* and *abortus* has been investigated and the results are tabulated in Table 1 Samples of six different lots of aureomycin were tested The antibiotic, dissolved in 0.85 per cent saline, was added to Trypticase Soy Agar (B B L, pH 7.0) while the latter was still fluid at a temperature of 50°C Plates were poured immediately and, after cooling, they were inoculated directly with a forty-eight hour broth culture of the test strain

It will be noted in Table 1 that the six strains of *Brucella suis* were completely inhibited after forty-eight to seventy-two hours of incubation by 0.25 to 0.5 micrograms of aureomycin per milliliter of medium

<sup>1</sup> The aureomycin used was supplied by the Lederle Laboratories Division, American Cyanamid Company

<sup>2</sup> These investigations were partially supported by grants received from Abbott Laboratories, Eli Lilly and Company, Lederle Laboratories Division, American Cyanamid Company, Parke, Davis and Company, and the Upjohn Company

Despite the known instability of this antibiotic (2, 3, 4, 5) in neutral or all saline media, complete inhibition of growth of relatively heavy inocula was still present after seven days when the concentration of the drug was 2.0 to 4.0 micrograms. Strains of *Brucella abortus* were somewhat more variable in their reaction to aureomycin and were inhibited by 0.25 to 2.0 micrograms per milliliter after forty-eight to seventy-two hours incubation. When observed after seven days incu-

TABLE 1  
*Sensitivity of Brucella to Aureomycin*

PATIENT	TYPE	SOURCE	SENSITIVITY* OF BRUCELLA TO VARIOUS SAMPLES OF AUREOMYCIN IN MICROGRAMS/MILLILITER											
			PERIOD OF INCUBATION											
			48 and 72 hours						7 days					
			1†	2	3	4	5	6	1	2	3	4	5	6
2	<i>Brucella suis</i>	Case #4	0.5	0.5	0.5	1.0	0.5	0.5	2.0	2.0	2.0	4.0	2.0	2.0
5	"	Case #5	0.5	0.5	0.5	0.5	0.5	0.25	2.0	2.0	2.0	2.0	2.0	2.0
6	"	Case #3	0.25	0.25	0.25	0.25	0.25	0.25	2.0	2.0	2.0	2.0	2.0	2.0
8	"	Case #1	0.5	0.5	0.5	0.5	0.5	0.5	2.0	2.0	2.0	2.0	2.0	2.0
9	"	Human	0.5	0.5	0.5	0.5	0.5	0.25	2.0	2.0	2.0	2.0	2.0	2.0
10	"	"	0.5	0.5	1.0	1.0	0.5	0.5	4.0	4.0	4.0	4.0	4.0	4.0
1	<i>Brucella abortus</i>	"	1.0	1.0	1.0	1.0	1.0	1.0	4.0	4.0	4.0	4.0	4.0	4.0
7	"	"	0.5	1.0	0.5	1.0	0.5	1.0	2.0	4.0	4.0	4.0	2.0	4.0
11	"	"	0.25	0.25	0.25	0.25	0.25	0.25	4.0	4.0	4.0	4.0	4.0	4.0
14	"	"	1.0	1.0	1.0	1.0	1.0	1.0	4.0	4.0	4.0	4.0	4.0	4.0
19	"	"	2.0	2.0	2.0	2.0	2.0	2.0	4.0+	4.0+	4.0	4.0+	4.0	4.0

\* Sensitivity = lowest concentration at which no growth was observed

† + = growth at the highest concentration employed

11 = Lederle Laboratories Lot No. 7-7854

2 = " " " 7-8020A

3 = " " " 7-8071A

4 = " " " 7-8071B

5 = " " " 7-8254

6 = " " " 7-8411

bation, four of the *Brucella abortus* strains were completely inhibited by 2.0 to 4.0 micrograms of aureomycin per milliliter but one strain was resistant to the latter concentration, which was the highest level tested.

Inasmuch as the strains of brucella were so sensitive to aureomycin, and the response obtained with the first patient was so promising, other patients ill with acute brucellosis were treated. Preliminary observations in experimental animals and humans had shown that the toxicity of this antibiotic was relatively low (2, 6, 7, 8, 9).

## CASE PRESENTATIONS

*Case #1* P T, a 45 year old fireman in a local meat processing plant, was hospitalized<sup>3</sup> on February 14, 1948 because of chills and fever of two weeks' duration

The patient had always been in good health until two weeks before admission when he experienced occipital headache, weakness, chills and fever. Chills occurring in the afternoon continued and, five days before admission, sulfadiazine was prescribed with some diminution in these symptoms. However, feverishness and drenching night sweats persisted. Two days before admission, an agglutination test was reported as significantly positive for brucella antibodies and he was referred to the hospital.

The patient was a well-developed and well-nourished male who was not in acute distress. His temperature was 100°F and the skin of the upper chest and face was flushed. The heart and lungs were normal. No masses or viscera were palpable on abdominal examination. No petechial or other eruptions were present.

Urinalysis and blood chemical examinations were normal. The hemoglobin was 13.2 grams, the total leukocyte count was 13,200 with a differential of polymorphonuclearleukocytes, 81 per cent and mononuclear cells, 19 per cent. The corrected sedimentation rate was 41 millimeters (Wintrobe).

Blood cultures were repeatedly and promptly positive for *Brucella suis* in three different laboratories. He was treated with 6 grams of sulfadiazine per day for twenty-three days and 3.0 grams of streptomycin intramuscularly daily for twelve days. Slight symptomatic improvement was noted but blood cultures were repeatedly positive during the period of combined drug therapy and subsequent to its termination. A trial with polymyxin D<sup>10</sup> was then deemed warranted and he was treated with 7.5 milligrams per kilogram of body weight intramuscularly daily for ten days. During this treatment his blood cultures promptly became negative and remained negative for one month. His general condition improved, although, fever up to 100°F (R) persisted. The patient was discharged on April 1, 1948 and was followed at weekly intervals.

He was readmitted to the hospital on May 3, 1948 because of recurrence of chills, fever, headache, malaise and night sweats during the preceding week. Blood cultures obtained at weekly intervals had been sterile until April 26, 1948 when *Brucella suis* was again cultured.

On physical examination the patient appeared ill. His temperature was 102°F, pulse 96 per minute and blood pressure 120/80. The skin was flushed, warm and moist. Erythema of the face, neck and upper thorax was marked. Petechial lesions were present on one toe and several fingers. Funduscopic examination showed a white area in the region of the right fovea. This was interpreted by the ophthalmological consultant as a "manifestation of a previous hemorrhage, prob-

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<sup>3</sup> Cases #1, 3 and 5 were admitted to Sinai Hospital, Baltimore, Maryland where they were on the service of Dr. Milton Sherry, Physician-in-Chief.

ably of embolic nature" The lungs were normal to percussion and auscultation The heart was not enlarged but a soft systolic murmur was intermittently audible at the cardiac apex His liver was enlarged two fingerbreadths below the right costal margin but splenomegaly was not detected Clubbing of the fingers and toes was present

The total leukocyte count was 3,200 with a differential of polymorphonuclear-leukocytes 79 per cent and mononuclear cells 21 per cent, hemoglobin was 13.4 grams The corrected sedimentation rate was 22 millimeters (Wintrobe) Urinalyses, blood chemical determinations, liver and kidney function tests were normal Agglutination tests for brucella ranged in titer from 1:5,120 to 1:20,480, for

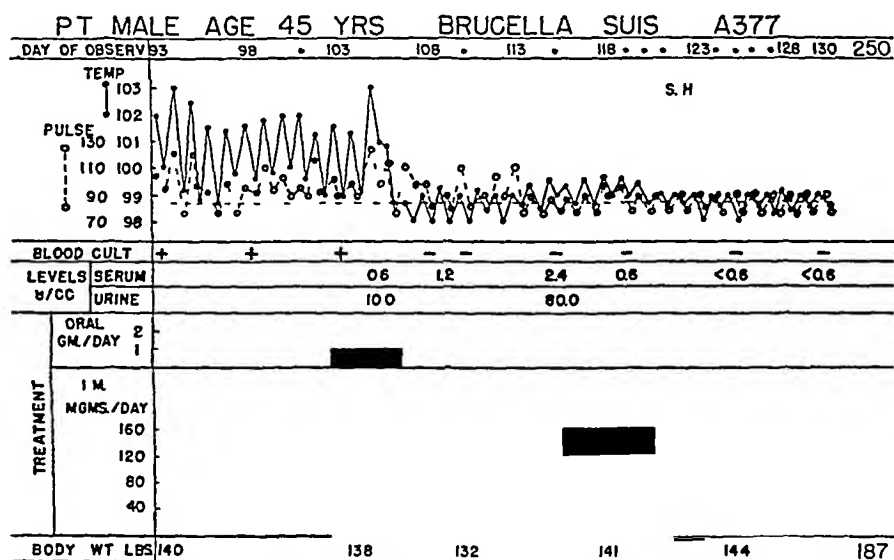


FIG 1

tularemia the titer was 1:320 Typhoid O and H and paratyphoid A and B agglutination tests were negative A determination of opsonins for brucella showed a marked increase of phagocytosis in the presence of the patient's serum when compared to that of a normal control

During the ten days prior to initiating treatment with aureomycin three consecutive blood cultures, positive for *Brucella suis*, were obtained The last of these cultures was taken immediately before treatment Since oral preparations of aureomycin were not available, this patient received crude aureomycin (lot No 205-214) in improvised capsules A total oral dose of 0.5 gram twice daily was administered for four days This dose was supplemented by intramuscular aureomycin hydrochloride Forty milligrams were administered intramuscularly every six hours for approximately twenty days During this entire period he received a total of 6.7 grams of the antibiotic Moderate nausea and anorexia were noted

while the patient was receiving oral crude aureomycin. Local tenderness and induration resulted from the intramuscular injections even when they were given in 1 per cent procaine solution.

The patient's temperature became normal within three days after aureomycin therapy was initiated. The liver regressed progressively and no new petechiae or emboli were observed. Eight blood cultures taken during the fifty days of hospitalization, concomitant with and following aureomycin therapy, were sterile. To date, eight months have elapsed since therapy with aureomycin was discontinued. The patient has remained well and has gained forty pounds in weight. He has returned to his former occupation and has performed his duties without difficulty.

*Case #2*<sup>4</sup> P S, a 48 year old farmer, was hospitalized on July 26, 1948 with the complaints of progressive weakness of three years' duration and intermittent fever.

The patient had been in good health until three years ago, at which time he began to complain of weakness and easy fatigability. Associated with this loss of energy, he experienced night sweats and frequent headaches. The fatigue and exhaustion had increased progressively, with exacerbations and remissions during the course of the next three years, accompanied by periodic fevers. One month prior to entry into the hospital, the patient again began to have chilly sensations and fever. An agglutination test for brucellosis was positive in a dilution of 1:320 at this time. He was given penicillin for several days and appeared to show some clinical improvement. However, his fatigue and weakness continued up to the point of complete exhaustion during the next fortnight. Four days prior to admission, his temperature rose to 104°F and ranged between 102°F-104°F until the time of admission.

Past history revealed that the patient had definite contact with Bang's disease in cattle. Three years prior to admission, there had been an outbreak of contagious abortion among his cattle. Despite this he continued to drink raw milk freely.

The patient was a lethargic, disoriented white male who appeared ill. Both his physical and mental reactions seemed unduly sluggish. The temperature was 102°F, pulse 96, respirations 20 and the blood pressure was 100 systolic, 90 diastolic. Except for occasional basilar rales in the posterior lung fields, the physical examination was normal.

His hemoglobin was 15 grams with a hematocrit of 46, his white cell count was 6,000 with 62 per cent neutrophils, 36 per cent lymphocytes and 2 per cent monocytes. Repeated urinalyses were normal. The sedimentation rate was 38 millimeters per hour. Agglutination tests employing *B. tularensis*, *B. paratyphosa* A and B, *B. typhosa* and *B. proteus* OX<sub>19</sub> as antigens were all negative. A blood culture taken at the time of admission was positive for gram-negative coccobacilli after ten days. These were identified as brucella organisms (abortus) by Dr. Carl

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<sup>4</sup> The authors are indebted to Dr. Sidney Ross, Children's Hospital, Washington, D. C. for his observations and the collection of data in this case.

Larson at the National Institute of Health Agglutination tests for *Brucella abortus* were positive in a dilution of 1:1,280

On the day following admission, oral therapy with aureomycin was begun During the first twenty-four hours, the patient received 200 milligrams every two hours For the following thirteen days the dose was 200 milligrams every four hours The patient's temperature fell to normal by lysis within seventy-two hours He remained afebrile during the remainder of his hospital course Concomitantly, a striking clinical improvement was noted He began to feel considerably stronger and his sensorium cleared within five days after the initiation of therapy There was a noteworthy improvement in appetite and vigor, and within ten days after aureomycin treatment had been begun the patient was ambulatory Following therapy, seven blood cultures were obtained during a twelve day period and all

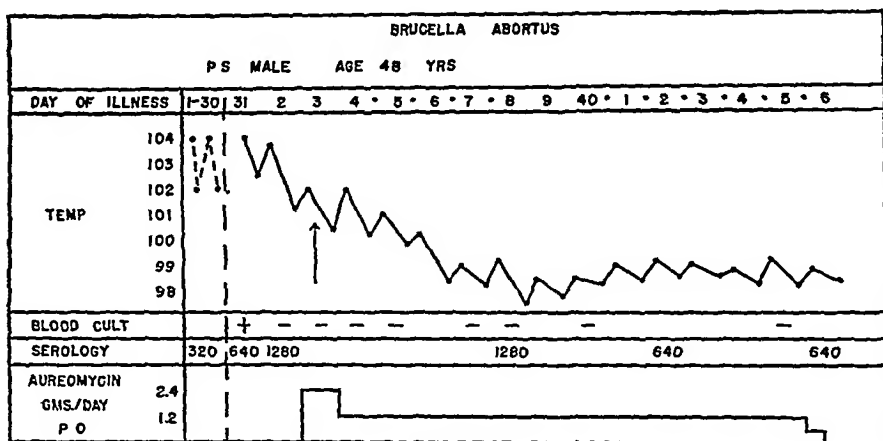


FIG 2

remained sterile No marked change was noted in the agglutination titer with brucella during the period of treatment, four agglutination tests taken at intervals were positive in dilutions of 1:640 to 1:1,280

Therapy with aureomycin was maintained for fourteen days During this time, the patient received a total of 18 grams of the antibiotic It was well tolerated and no reactions were observed He was discharged on his sixteenth hospital day and three months later was still in good health

Case #3 G S, a 47 year old male laboratory worker, was first hospitalized on July 21, 1948 with the complaint of weakness and headache for three weeks

The patient was employed as a laboratory worker and handled the cultures of *Brucella suis* obtained from P T (Case #1) for the five preceding months His fellow worker was J K (Case #5) Three weeks before admission, he noted malaise, profuse perspiration, temperature of 102°F, headache, weakness and anorexia He was given sulfadiazine without relief of his symptoms and his fever





mentation rate was 38 millimeters (Wintrobe) Urinalyses, chemical examinations of the blood, liver and renal function tests were all normal Agglutination tests for brucella ranged in titer from 1 640 to 1 10,240 Agglutination tests employing typhoid, paratyphoid A and B and proteus antigens were all negative A determination of opsonins for brucella revealed a marked increase of phagocytosis, over that of a normal, in the presence of the patient's serum

In the two days prior to treatment with aureomycin three consecutive blood cultures, subsequently positive for *Brucella suis*, were obtained The last of these cultures was taken immediately before treatment Aureomycin was administered orally 200 milligrams every hour for three doses initially and thereafter, 200 milligrams every two hours for six days It was then changed to 200 milligrams every three hours for eight days Aureomycin administered by the intramuscular route was used as a supplement in doses of 40 milligrams given every six hours during the first five days The drug was well tolerated The patient became afebrile within two days and all symptoms had disappeared by the fourth day of treatment The spleen decreased progressively in size and was no longer palpable after one week of therapy Aureomycin was discontinued at the end of two weeks Six blood cultures taken during the two weeks while on treatment were all sterile He was discharged from the hospital eighteen days after the initiation, and four days after completion of aureomycin therapy When seen four months later, he was asymptomatic, afebrile and working He had gained twelve pounds in weight

Case #4 W G, a 37 year old engineer, was hospitalized<sup>5</sup> on October 10, 1948 with the complaint of fever and chills of three weeks' duration

Two months prior to admission, while visiting on a farm in Florida, the patient drank unpasteurized milk He remained well until three weeks before entry when he noted generalized weakness, anorexia, malaise, chills and fever which was not influenced by quinine medication An agglutination test at this time was positive for undulant fever Past history and family history were non-contributory

The patient was well developed and well nourished He appeared acutely ill The temperature was 102°F, pulse 100, respiration 20 A diffuse erythema was present over the face and neck The skin was warm and moist Moderate, tender, generalized adenopathy was present The liver and spleen were palpable two fingerbreadths below the costal margin There was slight clubbing of the fingers and toes A flame shaped subungual hemorrhage was present on the fourth finger of the left hand The remainder of the physical examination was normal

Laboratory studies of the blood showed the hemoglobin to be 15 grams with a white cell count of 16,400 and 68 per cent polymorphonuclearleukocytes and 32 per cent mononuclear cells The sedimentation rate was 0 Urinalyses, blood chemical studies and liver function tests were all normal The agglutination titer for brucella ranged from 1 5,120 to 1 10,240 Agglutination tests for typhoid and

<sup>5</sup> Case #4 was admitted to the Church Home and Hospital, Baltimore, Maryland where he was on the service of Dr Z Morgan

paratyphoid A and B were negative. Determination of opsonins for brucella revealed a marked increase of phagocytosis in the presence of the patient's serum. Three consecutive blood cultures taken on the second and third days following admission were positive for *Brucella suis* in two different laboratories.

On the third day of hospitalization, antibiotic therapy was started. Streptomycin 2 grams intramuscularly and sulfadiazine 6 grams orally per day were administered to the patient without manifest change in his condition. After four days, the sulfadiazine and streptomycin were discontinued. The patient was then given aureomycin by mouth, 200 milligrams every hour for three doses, and this was followed by 200 milligrams every two hours for one day. Thereafter, 200 milligrams was administered every four hours for twelve days. The following four days, he received 200 milligrams every two hours. He also was given supplements of aureomycin by the intramuscular route consisting of 40 milligrams every six hours for the first day, and 40 milligrams every twelve hours for ten days. During the entire period he received a total of 18.8 grams of aureomycin. The drug was well tolerated and no nausea or vomiting occurred. Within seventy-two hours after the initiation of therapy, the temperature was normal and the patient became asymptomatic. The spleen and liver decreased progressively in size and were no longer palpable two weeks after the beginning of treatment. The patient was discharged from the hospital seven days after therapy was discontinued. Seven blood cultures taken during the eight weeks concomitant with and following therapy have remained sterile. When seen two months following his discharge from the hospital, the patient had remained well. He had gained ten pounds in weight.

Case #5 J. K., a 53 year old male laboratory worker, was hospitalized on October 22, 1948 complaining of fatigue of two months' duration.

The patient had worked for years as a laboratory helper. He handled the cultures of *Brucella suis* obtained from P. T. (Case #1) four to eight months previously. His fellow worker was G. S. (Case #3). Two months prior to admission to the hospital, he developed malaise, fever, chills and night sweats. This was associated with moderate anorexia and weight loss. One month before entry, he noted the onset of persistent, sharp pains in the left upper quadrant. Ten days before admission, he complained of persistent frontal headache. Because of these symptoms and positive agglutination tests for brucellosis, he was hospitalized. Past history and family history were non-contributory.

The patient was fairly well developed, well nourished and had a marked kyphoscoliosis. He appeared chronically ill. His temperature was 101°F, pulse 90, and blood pressure 120/80. The skin was warm and moist. There was slight epigastric and left upper quadrant tenderness. The liver was firm, smooth and non-tender with the edge palpable four fingerbreadths below the right costal margin. The spleen tip was firm and blunt and palpable four fingerbreadths below the left costal margin. There was no generalized adenopathy and the physical examination was otherwise normal.

His total leukocyte count was 5,500 with a differential of 72 per cent neutro-

philes, 24 per cent lymphocytes, and 4 per cent monocytes. The hemoglobin was 13.8 grams. The corrected sedimentation rate was 22 millimeters (Wintrobe). Urinalyses, chemical examinations of the blood and liver function tests were all normal. Agglutination tests for brucella ranged in titer from 1:160 to 1:10,240. The results of other agglutination tests were: typhoid O negative, typhoid H 1:640, paratyphoid A and B 1:160.

During the twenty-one days prior to aureomycin treatment, five consecutive blood cultures positive for *Brucella suis* were obtained from venous, arterial blood and bone marrow. Four days following the last culture, aureomycin was administered. Unfortunately, the culture drawn the morning before treatment was begun has been lost. The dosage schedule utilized was 250 milligrams every hour for three doses and thereafter 250 milligrams every two hours for eight days. The drug was then continued at a dosage of 250 milligrams every six hours for seven days. Aureomycin given by the intramuscular route in doses of 40 milligrams every eight hours for two days and every twelve hours for two additional days was employed to supplement the oral drug. During this sixteen day period, he received a total of 35.25 grams of aureomycin. The drug was well tolerated but mild nausea and frequent unformed stools occurred on the seventh day of therapy. This was relieved with an aluminum hydroxide preparation and disappeared on continued treatment.

The patient gradually regained his strength and appetite. Night sweats disappeared and within a week he was asymptomatic. His spleen and liver gradually decreased in size. Aureomycin was discontinued after sixteen days of therapy and the patient was discharged one week later. Four blood cultures drawn in the three weeks following the beginning of treatment were sterile. He has remained well for the past two months and has gained five pounds.

#### SUMMARY OF CLINICAL FINDINGS

Four of these five patients were treated on the eighteenth to the one hundred and third day of their disease. The remaining patient had a three year history characterized by periodic exacerbations and remissions of illness. He was in the thirtieth day of his latest relapse when treated. All patients were males ranging in age from thirty-seven to fifty-three years. All had a history of contact with infected or potentially infected material. Patient #1 worked in a meat packing plant. Patients #3 and #5 as laboratory workers handled the cultures of *Brucella suis* obtained from the blood of Patient #1. Patient #2 was a farmer who drank raw milk and had repeated contact with Bang's Disease in cattle. Patient #4 drank raw milk while vacationing on a farm.

The illness in these five patients was characterized by marked fatigue

which was accompanied or followed by fever, night sweats, muscle pains, anorexia and headache Four patients complained of shaking

TABLE 2  
*Summary of Clinical and Laboratory Data*

PATIENT	1	2	3	4	5
Initials	P T	P S	G S	W G	J K
Age	45	48	47	37	53
Sex	M	M	M	M	M
Occupation	Meat packing plant	Farmer	Laboratory Worker	Engineer (vacation on farm—raw milk)	Laboratory worker
Symptoms					
Fatigue	++++	++++	++++	+++	++++
Sweats	++++	++++	++++	++	++++
Myalgia	++++	+++	+++	++++	+++
Anorexia	++++	+++	++++	+++	++
Headache	++++	++++	++++	++	+
Chills	++++	+	0	++++	++
Arthralgia	+++	0	++++	0	0
Somnolence	0	++++	0	0	0
Signs					
Fever (°F)	101-103°	102-104°	99 <sup>a</sup> -103 <sup>a</sup>	100-103°	99-101°
Erythema	++++	0	++++	++++	0
Weight loss	++++	+	+++	++	++
Hepatomegally	+++	0	+	++	++
Splenomegally	0	0	+++	+	+++
Petechial	+++	0	+	++	0
Clubbing	+++	0	0	+	0
Laboratory					
Blood cultures					
Pre treatment	13 positive (Br suis)	1 positive (Br abortus)	3 positive (Br suis)	3 positive (Br suis)	5 positive (Br suis)
Post treatment	8 negative	7 negative	6 negative	7 negative	4 negative
Agglutination titer	5,120-20,480	320-1,280	640-10,240	5,120-10,240	160-10,240
Previous brucella vaccine	None	None	None	None	None

chills Only two patients experienced joint pains Patient #2 was lethargic and disoriented prior to treatment and may well have had a meningo-encephalitis (11, 12) In all patients the fever was of a swinging type with marked diurnal variation In general, temperatures were lowest in the morning and rose in the afternoon and evening All

had experienced moderate to severe weight loss. The liver was slightly to markedly enlarged in four cases. In three the spleen was definitely palpable. Erythema of the upper thorax, neck and face, associated with dilated small subcutaneous blood vessels was present in three patients. Petechial and embolic phenomena were most marked in Patient #1. He was observed to have transient, subungual splinter hemorrhages in the fingers, painful erythematous nodules at the tips of fingers and toes, and an organizing hemorrhage near the right macula. This patient had developed definite clubbing of fingers and toes. A soft systolic murmur was intermittently audible at the mitral area. Although splenomegaly was not detected, the diagnosis of an endocarditis due to *Brucella suis* was considered as a distinct possibility (11, 12). Two other patients showed evidence of petechial phenomena. One of these had minimal clubbing of the fingers.

#### SUMMARY OF LABORATORY DATA

*Brucella* organisms were isolated from the blood of all five patients prior to the institution of aureomycin therapy. All cultures taken during or following treatment were sterile. Four of the isolated strains were classified as *Brucella suis* and one as *Brucella abortus*. In Patients #1 and #4 cultures of *Brucella suis* were obtained simultaneously but independently in several different laboratories. Prior to therapy with aureomycin, Patient #1 had had thirteen positive blood cultures. Three of these were obtained within ten days and one immediately before administration of aureomycin. Eight blood cultures during the six weeks following treatment were sterile. *Brucella abortus* was cultured from the blood of Patient #2 two days before initiation of therapy. Seven blood cultures taken during the following two weeks were sterile. From Patient #3 *Brucella suis* were grown from three consecutive blood cultures obtained during the seventy-two hour period before treatment was initiated. Six cultures taken during the following two weeks were sterile. Three blood cultures containing *Brucella suis* were obtained from Patient #4 on the fourth and fifth days preceding aureomycin therapy. During the two months following treatment seven cultures were sterile. Cultures of venous blood, arterial blood and bone marrow obtained from Patient #5 all contained *Brucella suis*. This patient had four cultures containing *brucella*

organisms drawn during the five days prior to initiation of aureomycin therapy. Four cultures obtained in the three weeks during and following treatment were sterile.

Agglutination titers for brucella in the serum of all patients reached 1:1,280 or higher. Three cases had titers of 1:10,240. One patient had agglutinins for brucella in serum diluted 1:20,480. The remaining patient had a titer of 1:1,280. None of these patients had ever received brucella vaccine or brucellergin skin tests. Tests for opsonins were performed with the serum of three of these patients. A gradual increase in the phagocytosis of brucella organisms exposed to leukocytes in the presence of these sera was noted when compared with normal control sera. Four of the cases showed a mild leukopenia prior to treatment. One patient had moderate leukocytosis. A relative lymphocytosis was observed in all patients at some time during the course of their illness. Erythrocyte sedimentation rates were elevated in four of the five patients. Three of the four cases showed a diminution of the sedimentation rate during or following therapy with aureomycin.

Repeated urinalyses, renal and liver function tests were normal prior to treatment and remained within normal limits during and following the administration of aureomycin. Determinations of blood urea nitrogen, fasting blood sugar, cholesterol, uric acid, creatinine, albumin, globulin, total protein, bilirubin, alkaline phosphatase, thymol turbidity, cephalin flocculation and icteric index in four of these cases remained within the normal range. Although these studies were incomplete in one case, there was no evidence to suggest any abnormality.

#### SUMMARY OF TREATMENT

An arbitrary oral dosage of 2,400 to 3,000 milligrams of aureomycin (30 to 60 milligrams per kilogram of body weight) per day was initially administered to all patients except the first. A "priming" dose of 200 or 250 milligrams was generally given every hour for three doses. This same dose was then given every two hours for a variable period ranging from one to eight days. The antibiotic was then continued in 200 or 250 milligram doses every three or four hours for a total period of approximately fourteen days. Patient #1 was treated before an oral preparation of aureomycin was available and he received improvised capsules containing 500 milligrams of the crude drug. The latter cap-

sules were given twice a day for four days In all patients except the second, supplemental aureomycin was administered by the intramuscu-

TABLE 3

*Brucellosis*

PATIENT			TREATMENT				RESULT**
No	Initials	Wt	Previous treatment	Day of dis ease	Aureomycin		
					Total drug	Dosage schedue	
		lb			gm		
1	P T	135	Streptomycin 3 gm per day for 11 days Sulfadiazine 6 gm per day for 23 days Poly myxin 420 mgs I M for 10 days	103	6 7	500 mg q 12 h x 4 days oral 40 mg q 6 h x 20 days I M	Afebrile in 72 hours Asymptomatic in 6 days No re lapse in 8 months
2	P S	185	Penicillin	30*	18 0	200 mg q 2 h x 1 day oral 200 mg q 4 h x 13 days oral	Afebrile in 3 days Asymptomatic in 5 days No re lapse in 3 months
3	G S	123	Sulfadiazine	72	27 0	200 mg q 1 h x 3 doses oral 200 mg q 2 h x 6 days oral 200 mg q 3 h x 8 days oral 40 mg q 6 h x 5 days I M	Afebrile in 48 hours Asymptomatic in 4 days No re lapse in 4 months
4	W G	171	Streptomycin 2 gm per day and Sulfadiazine 6 gm per day for 4 days	18	18 8	200 mg q 1 h x 3 doses oral 200 mg q 2 h x 1 day oral 200 mg q 4 h x 12 days oral 40 mg q 6 h x 1 day I M 40 mg q 12 h x 10 days I M	Afebrile and asymptomatic in 72 hours No relapse in 2 months
5	J K	110	None	65	35 25	250 mg q 1 h x 3 doses oral 250 mg q 2 h x 8 days oral 250 mg q 4 h x 7 days oral 40 mg q 12 h x 2 days I M 40 mg q 8 h x 2 days I M	Asymptomatic in 7 days No relapse in 2 months

\* + 3 year history

\*\* Patients 1 3 4 and 5 have been seen since this manuscript was submitted They were well and examination revealed no evidence of recurrence of their disease Blood cultures obtained have remained sterile to date (1/27/49)

lar route Injections of 40 milligrams of aureomycin dissolved in 2 cc of a 1 per cent procaine hydrochloride solution were administered every



six, eight or twelve hours for four to seventeen days. The total amount of aureomycin employed varied from 6.7 to 35.3 grams and was administered orally and/or intramuscularly during a thirteen to twenty day period. Nausea following the administration of aureomycin by mouth was noted in two cases and was accompanied by vomiting in one. Except for patient #1, who received the crude aureomycin, this nausea subsided on treatment. Aluminum hydroxide preparations were successfully employed to counteract the local gastric irritation. Intramuscular injections of aureomycin dissolved in 1 per cent procaine solution caused considerable pain and induration at the site of injection. Suppuration was not noted. In one patient three to four unformed stools were noted daily on the seventh day of treatment. This patient was receiving a 250 milligram capsule every two hours. These symptoms subsided within two days when an aluminum hydroxide preparation was administered. The dosage of aureomycin had also been reduced to 250 milligrams every four hours.

#### COMMENT

Four of the above patients had high fever prior to aureomycin treatment. All became and remained afebrile within seventy-two hours after the onset of aureomycin therapy. Symptoms markedly diminished with defervescence. In every case headache, fatigue, night sweats, myalgia, chills, arthralgia and anorexia disappeared during the first week of treatment. When palpable, the liver and spleen progressively decreased in size under therapy. New embolic and petechial phenomena were not observed after aureomycin was administered.

All patients have returned to their former occupations. In each instance a gain in weight followed treatment. This has ranged between five to forty pounds. There have been no relapses. Three patients have been followed for three to eight months. The remaining two patients have remained afebrile and asymptomatic for more than two months. In all patients repeated blood cultures for brucella have remained sterile. It is to be noted that positive cultures for *Brucella suis* were obtained from Patient #1 while he was receiving three grams of streptomycin and six grams of sulfadiazine daily.

Although brucellosis is characterized by an erratic course marked by exacerbations and remissions, it is felt that the results recorded are

sufficiently clear to indicate that aureomycin exerts a beneficial influence in this disease. It is felt that this antibiotic is an effective chemotherapeutic agent for the treatment of undulant fever.

#### SUMMARY

Studies with multiple strains of brucella including both *Brucella abortus* and *Brucella suis* have indicated that these organisms are sensitive to the antibacterial action of aureomycin *in vitro*. 0.25 to 2.0 micrograms of aureomycin per milliliter of medium completely inhibited growth of eleven strains of brucella during the first seventy-two hours of incubation.

Five patients having blood cultures positive for brucella organisms have been treated with aureomycin. All became afebrile and were markedly improved within forty-eight to seventy-two hours after the initiation of therapy. Positive cultures were not obtained after treatment was begun. When palpable, the liver and spleen receded during therapy, and further evidence of active disease was not observed. Relapses have not been observed during periods of observation after treatment of from two to eight months. Weight gains of five to forty pounds have been noted during this period.

The only toxic reaction associated with oral administration of aureomycin was occasional, transient nausea. This was relieved by aluminum hydroxide preparations. The intramuscular injection of the drug, even when dissolved in 1.0 per cent procaine, produced local pain.

In all but one patient, the initial treatment consisted of 2,400 to 3,000 milligrams orally. Supplemental intramuscular injection of the drug was administered to four patients. The dosage was usually reduced when the patient became afebrile. Aureomycin therapy was continued empirically for approximately two weeks. Supplemental aureomycin injected intramuscularly, may not be necessary for the treatment of brucellosis.

The laboratory and clinical observations in these five cases of brucellosis would suggest that aureomycin is an effective chemotherapeutic agent in this disease.

#### ADDENDUM

As this paper was being submitted for publication, Spink, *et al* (J A M A 138: 1144, 1948) reported on the treatment with aureo-

mycin of *Brucella melitensis* infections with good immediate therapeutic results

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# EXPERIMENTAL KLEBSIELLA MENINGITIS TREATED WITH INTRATHECAL AND INTRAMUSCULAR STREPTOMYCIN<sup>1</sup>

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The discovery of agents effective in the treatment of the various bacterial meningitides introduced a question which is as yet unsettled, namely, "Is it necessary for the successful treatment of a meningeal infection to effect inhibitory concentrations of the chemotherapeutic agent in the cerebrospinal fluid?" In the absence of data bearing directly on this matter, clinicians have assumed such necessity (1, 2) and there are certain considerations invoked in support of this assumption. The concept of the infected area in meningitis, must include underlying nervous tissues as well as the cerebrospinal fluid and meninges (3-5). In the normal animal such substances as the bromide and iodide ions (6), and the sulfonamides (7) appear in equal concentrations in the brain and cerebrospinal fluid, and presumably in the meninges, when administered systemically. The ability of some chemotherapeutic agents to reach the infected area as judged from these data obtained in normal animals is thus reflected by their concentration in the cerebrospinal fluid. Some agents, such as penicillin and streptomycin, appear in the cerebrospinal fluid (1, 2), and in the brain (8, 9) in negligible concentrations after systemic administration. It was therefore thought that the capacity of these agents to cure meningitis would be poor unless they were injected directly into the cerebrospinal fluid (1, 2). The necessity for intrathecal injection of these antibiotics has recently been reconsidered because 1) such injections are toxic (10, 11) and 2) the use of large doses of antibiotics systemically in the presence of inflamed meninges brings about fairly high cerebrospinal fluid concentrations (9, 12).

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<sup>2</sup> Roche Fellow in Medicine and Experimental Therapeutics

Previous animal studies of the treatment of meningitis have not answered this question because of the low virulence of the infection employed (13, 14). Clinical reports do not permit a conclusion because of the small numbers of cases treated (15, 16), concomitant use of sulfonamides (10, 17) or failure to use comparable controls (18, 19). Therefore, a meningitis in experimental animals was sought which would be suitable for studies of the comparative effectiveness of intramuscular, intrathecal and combined intramuscular and intrathecal administration of a "non-diffusible" antibiotic.

TABLE 1

*Effect of Culture Dilution upon Survival Time of 68 Cats Infected Consecutively with K pneumoniae Intracisternally*

DILUTION OF CULTURE	NUMBER OF ANIMALS	AVERAGE HOURS OF SURVIVAL
10 <sup>-1</sup>	4	16
10 <sup>-2</sup>	13	19
10 <sup>-3</sup>	6	27
10 <sup>-4</sup>	3	33
10 <sup>-5</sup>	4	35
10 <sup>-6</sup>	35	65
10 <sup>-7</sup>	3	80

#### MATERIALS AND METHODS

The meningitis produced in cats by Weed et al (20) using *B mucosus capsulatus*, was the most virulent of those found in the literature. It was assumed that their organism was a *Klebsiella*, and a trial with a *Klebsiella* strain produced a satisfactory infection (table 1).

*Klebsiella Pneumoniae Infection* The cats were infected with *Klebsiella pneumoniae*, strain BE. Stock cultures were grown in trypticase-soy-phosphate broth. Virulence was maintained by passage through cats, 48 hours before each experiment. For passage 10 ml of a 10<sup>-2</sup> dilution in modified Ringer's solution (21), of a 6-hour subculture, was given intracisternally, after the withdrawal of a similar amount of cisternal fluid. A culture of cisternal fluid was obtained soon after death, incubated for 6 hours at 37.5° C and then kept at 6° C until the day of the experiment.

All untreated cats have died of such infection with positive cultures of the cisternal fluid. Death occurred after the introduction of as few

as 50 organisms. The survival time in the untreated cats was roughly proportional to the culture dilution (table 1). No histological studies were done in this laboratory, although Weed et al. (20) have discussed the pathology of a similar meningitis in their monograph. The proof that this infection is a meningitis rests also on the fact that at the time treatment was started, the cisternal fluid was cloudy and contained innumerable polymorphonuclear leucocytes. In addition, the cultures always became positive and frequently the untreated cats developed the clinical manifestations of meningeal irritation.

For the streptomycin experiment, one ml of the stock culture was added to nine ml of TSP broth and incubated for exactly 6 hours. One ml of a  $10^{-6}$  dilution of this subculture, in modified Ringer's solution, was used for the infection. This inoculum contained an average of 2000 organisms. The first and last cats infected in each experiment were saved as untreated controls. A few of the controls received cisternal injections of sterile Ringer's solution, but these cats died more quickly than usual, so the procedure was not made part of the routine.

*Streptomycin.* A single lot (Pfizer P4718) of amorphous streptomycin sulfate was used for treatment. Chemical assay (22) showed that 77 per cent of this material was streptomycin base. Dosage was calculated in terms of streptomycin base per kilogram of cat. Dilutions were made in sterile modified Ringer's solution. The dose of intracisternal streptomycin was the same for all cats, while the intramuscular dosage varied. The intramuscular drug was given once a day for three days, starting exactly three hours after infection, 50 per cent of the total dose was given in the first injection, and 25 per cent in each of the 2nd and 3rd injections. Intrathecal streptomycin was given in two equal doses at 3 hours and at 24 hours after infection. The intrathecal drug was made up in a dilution of 4 mg per ml. 0.2 to 1.0 ml were injected intracisternally in a total dose of 2 mg/kg after the removal of the same volume of cerebrospinal fluid. All the cats were under chloroform anesthesia during the infecting procedure, and, excluding the controls, during the first and second treatments. The cats on intramuscular dosage alone therefore were anesthetized as often as the intrathecal group but did not have additional intracisternal punctures. Sufficient fluid for streptomycin levels could not be obtained.

*Cats* The cats constituted an extremely heterogenous group as one cannot obtain cats of the same age, size, and infectional and nutritional backgrounds. Efforts were therefore made to avoid elements of selection. This was accomplished by two maneuvers. 1) The cats were assigned to a given dosage schedule by means of the shuffled card

TABLE 2

*Effect of Route of Administration of Streptomycin upon Survival Rate of Cats with K pneumoniae Meningitis*

Infection Intracisternal, 10 ml of  $10^{-6}$  dilution of 6-hr subculture of *K pneumoniae*, strain BE, containing 2000 organisms

Streptomycin Intramuscular, 50% of total dose at 3-hrs, 25% at 27 and 50 hrs after infection Intracisternal, total dose constant at 2 mg/kg, 50% at 3 hrs and 50% at 27 hrs after infection

TOTAL DOSE OF INTRAMUSCULAR STREPTOMYCIN	UNTREATED CONTROLS	PROPORTION* OF SURVIVORS		
		Intramuscular	Intracisternal	Intramuscular and Intracisternal
mg/kg				
64		7/7		6/7
32		2/7		5/8
16		3/9		5/7
8		2/11		7/7
4		1/8		5/8
2		2/9		
0	0/19		6/9	

\* Numerator represents animals surviving 21 days with negative cultures of blood and cisternal fluid. Denominator represents total animals infected, it excludes animals infected but dying within 21 days with negative cultures. These amount to intramuscular streptomycin—3 cats at 32 mg/kg, 1 at 4, 1 at 2, intracisternal—1 cat, intramuscular and intracisternal—1 cat at 32 mg/kg, 3 at 16, 2 at 8 and 2 at 4. The difference between numerator and denominator therefore represents animals dying with positive cultures CSF or blood or both.

method, 2) Each single experiment was devised so that it contained each of the 12 dosage schedules shown in the final table. This type of experiment was repeated at weekly intervals, and the results pooled in table 2.

*Follow-up* In the first series of experiments the surviving cats were held for at least 21 days after infection, then sacrificed and cultures obtained of the cisternal fluid and heart blood. Similar cultures were obtained from cats dying before 21 days. Eighty per cent of the ani-

mals dying with positive cultures did so in the first week. One cat was found to have a positive blood culture, but negative spinal fluid cultures on the 21st day. Deaths sometimes occurred with negative blood and spinal fluid cultures. Such deaths are noted separately in the table.

### RESULTS

The results of the streptomycin treatment of this meningitis are shown in table 2. With combined intramuscular and intracisternal streptomycin treatment, the rates of survival were constant and of the same order of magnitude as with intrathecal drug alone. At each dosage level, except the highest tested, there was a consistent trend in favor of the combined therapy when compared with the group on

TABLE 3

*Virulence Titration of K. pneumoniae Meningitis in Cats Preceding Second Series of Experiments*

DILUTION OF CULTURE	NUMBER OF ANIMALS	SURVIVAL TIME IN HOURS
$10^{-2}$	1	16
$10^{-3}$	1	16
$10^{-4}$	2	26
$10^{-5}$	2	42
$10^{-6}$	3	63

intramuscular treatment alone. This consistent difference between the two groups, when considered as a group difference, is statistically significant. With intramuscular drug alone, at dosages from 2 to 32 mg/kg, survivals were erratic, as though streptomycin diffusion occurred in some cats but not in others. When 64 mg/kg were given by the intramuscular route alone, 100 per cent survival was achieved. It seemed important to document further the high survival rate after intramuscular streptomycin alone and therefore a second series of experiments was undertaken.

### METHOD

The same procedures and materials were followed as outlined above. The *Klebsiella* was again obtained from Dr. H. J. White. After successive passage through 3 cats, a virulence test was run (table 3). Since virulence did not seem diminished, streptomycin experi-



ments were undertaken. The animals were separated into three groups—untreated controls, treated with intramuscular streptomycin alone, and treated with combined intramuscular and intrathecal streptomycin. The total intramuscular dosage was 128 mg/kg for all cats. The intrathecal dosage was the same as in the first experiment. The animals were followed for two weeks instead of three.

TABLE 4

*Effect of High Doses of Intramuscular Streptomycin Alone upon Survival Rate of Cats with Klebsiella pneumoniae Meningitis*

Infection: Intracisternal, 1.0 ml of  $10^{-6}$  dilution of 6-hr subculture of *K. pneumoniae* containing 2000 organisms

Streptomycin: Intramuscular, Total dose 128 mg/kg, divided into 3 doses, 50% at 3 hrs, 25% at 27 and 50 hrs after infection. Intracisternal, Total dose 2 mg/kg, 50% at 3 hrs and again at 27 hrs after infection.

	UN-TREATED CONTROLS	INTRA MUSCULAR STREP TOMYCIN	INTRA CISTERNAL & INTRA MUSCULAR STREP TOMYCIN
Total Infected	11	23	20
Survived 14 Days with Negative Cultures		13	10
Died in less than 14 Days with Negative Cultures		9	10
Died with Positive Cultures	11	1	0

## RESULTS

The effect of high intramuscular dosage is shown in table 4. A number of deaths occurred before the 14th day, because of an epidemic of pneumonitis. The entire experiment is shown, although conclusions should only be drawn from the survivors. Only one cat died with a positive culture, and this was of the blood only. It is clear that intramuscular drug alone, in large doses, can routinely bring about survival from the infection.

## DISCUSSION

It has not been possible in this study to examine directly the relationship between survival of the infected cats and the actual concentration of the streptomycin in the cerebrospinal fluid. Nevertheless, the data permit the inference that it is the streptomycin reaching

the cerebrospinal fluid that represents the ability of the drug to overcome the infection. In other words, this infection seems to be in equilibrium with the drug in the cerebrospinal fluid. This principle has been examined under rigid and therefore artificial conditions and its applicability to clinical meningitis can be determined only by direct examination in patients. Certain points must be made however, so that conclusions from these data will not be over-extended. One can only conclude that in the treatment of meningitis in patients, chemotherapeutic agents should continue to be used in such a manner as to insure their appearance in the cerebrospinal fluid in antibacterial concentrations. It should not be assumed that intrathecal injection of penicillin or streptomycin into the lumbar region is the best way of distributing the drug to the entire infected area. In general, the concentration of intrathecally administered drugs, such as bromides, is greatest at the site of injection, and in other areas diminishes in proportion to its distance from the point of injection (6). With penicillin, the concentration in ventricular fluid after lumbar injection is variable and sometimes negligible (23). On the other hand, when substances such as bromide or iodide anions (6) or salicylates (24) appear in the cerebrospinal fluid after systemic administration equal concentrations are found in all parts of the cerebrospinal fluid, even below a complete block. In the treatment of meningitis with drugs that are not readily transferred from the plasma to the cerebrospinal fluid, intrathecal injection in the lumbar area is probably an inefficient way of getting the drug into the infected area. It would seem more desirable to accomplish the transfer of drug from blood to cerebrospinal fluid. Efforts in this direction have been made by the use of caronamide and large doses of penicillin (25), and by the use of 12 million units daily of intramuscular penicillin alone (15). These methods would appear to accomplish transfer of penicillin to the cerebrospinal fluid in antibacterial concentrations. Whether such methods are as effective treatment of clinical meningitis as intrathecal injections must be subjected to cautious study in patients.

The toxicity of intrathecally administered antibiotics is another objection against the direct injection of these agents into the cerebrospinal fluid. However, one suspects that for any given concentration of the drug, the general toxicity would be the same whether the agent

reached the cerebrospinal fluid by direct injection or by transfer from the blood. The local toxicity of the antibiotic might be greater after intrathecal injection because the unreliable distribution from the lumbar route requires the administration of fairly large amounts of drug in order to achieve antibacterial concentrations throughout the cerebrospinal fluid. Thus, 20,000 units of penicillin given intrathecally in a volume of 10 ml may give a local concentration in the lumbar spinal fluid of  $> 700$  units/ml (23). Hence the problem again returns to finding a better method of distributing antibiotics throughout the infected area.

#### SUMMARY AND CONCLUSIONS

- 1) A highly virulent uniformly fatal meningitis has been induced in cats by intracisternal injection of *Klebsiella pneumoniae*.
- 2) Treatment by means of intrathecal, intramuscular, or combined administration of streptomycin, suggests that survival of the cats is governed by the amount of drug that reaches the cerebrospinal fluid.
- 3) Survival of the infected cats occurs routinely when large doses of streptomycin are given solely by the intramuscular route.
- 4) The possible clinical implications of these data are discussed, with emphasis on the deficiencies of the lumbar intrathecal route as a method of distributing the antibiotic to the infected area.

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# THE PREVENTION AND TREATMENT OF MOTION SICKNESS\*†

## I SEASICKNESS

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### 1 INTRODUCTION

Scientists have been puzzled by their inability to find a remedy for the disease known as motion sickness. No record to date is published of any drug which has satisfactorily prevented, allayed, or cured this unfortunate malady. Many investigators have studied motion sickness, especially during World War II, and many drugs have been used in an attempt to control its symptoms, but no drug that can be used liberally and repeatedly has been found. Drugs which were apparently successful often produced side effects as seriously handicapping as motion sickness itself. Careful study has proved hyoscine the most effective drug so far employed. Hollings, McArdle and Trotter (1) in 1944, on behalf of the Medical Research Council of Great Britain, studied the effect of a number of drugs on short trips to sea in minesweepers and trawlers. These trips were of two to six hours' duration. The side effects from hyoscine were such that they did not recommend repeated doses. Tyler (2) in 1946, under contract with the Committee on Medical Research of the United States, studied the influence of a placebo, body position, and medication on motion sickness. He showed that the psychogenic factors play little part in susceptible individuals. He placed a large number of men in small boats (amphibious operations) gave to half of them a placebo and to half no medication. Thirty-five per cent of each group became

\* This investigation was made possible by the Chief of Staff and the Surgeon General of the United States Army

Appreciation is expressed to the Transport Command, especially to Major C W Hodges, Lt J L Soelling and to the nurses, of the U S A T General Ballou

† The authors wish to express their appreciation to G D Searle & Co of Chicago, who prepared the drug Dramamine and the placebo for the investigation

\*\* From the Allergy Clinic of the Medical Department of the Johns Hopkins University and Hospital

ill in shore to shore operations In the untreated control group thirteen per cent had severe symptoms and in the placebo treated group fifteen per cent had severe symptoms The position which men occupied in small boats played a part in the number of ill men, as well as in the severity of symptoms In a crouching position thirty per cent became seasick, in the standing position eleven per cent became ill Ten per cent of the crouching men were very sick compared with two per cent of the standing men Medication was more effective than a placebo Barbiturates were very unsatisfactory, and hyoscine afforded an average of sixty per cent protection when placebo rates were as high as fifty-two per cent Noble, Sellers, and Best (3), in an investigation sponsored by the National Research Council of Canada in 1947, studied swing sickness in dogs and in man and reported that "while a correlation between swing sickness and sea or air sickness could be shown to exist it did not appear to be of high order" The belladonna alkaloids which they used to control symptoms produced three side effects which were objectionable hallucinations, excited states and mental cloudiness The mental cloudiness occurred most prominently with hyoscine, especially common with 0.8-1.2 mg It is of interest to note their comment that atropine, hyoscine and hyoscyamine are totally inactive in preventing motion sickness in dogs The authors state that attempts to make sea trials using large numbers of subjects "were ruined by fair weather" Lilienthal (4) reported the effect of hyoscine on airsickness He found that 0.6 mg administered orally 30-60 minutes before flight was a preventive of airsickness with insignificant side effects He did not report concerning the effect of hyoscine on combat troops when administration of the drug becomes necessary every few hours, as is the case when a landing operation must be carried on many miles from the base of origin

A compound thought to be more uniformly effective in the prophylaxis and treatment of motion sickness than any drug so far employed and without unpleasant side effects is the subject of this report In 1947 a new drug, Dramamine, was sent by the manufacturers\* to the Allergy Clinic of the Johns Hopkins University and Hospital for experimental investigation of its possible value in the control of sea

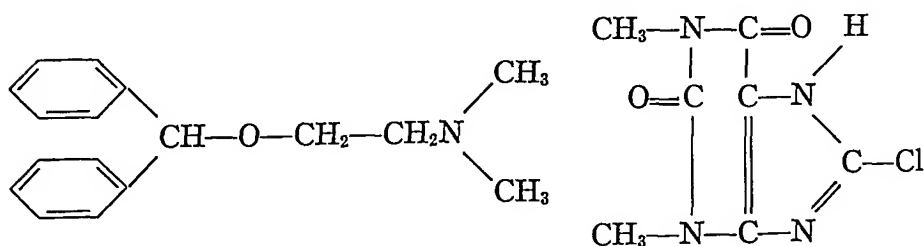
\* G. D. Searle & Co., Chicago, Illinois

fever and urticaria Among other patients, the drug was administered to a pregnant woman with urticaria who incidentally had suffered all her life from car sickness It was possible to control the car sickness of this patient at will A placebo failed repeatedly, but Dramamine gave complete relief if she took 50 mg a few minutes before she boarded a streetcar

Subsequent to this isolated clinical observation, other victims of car sickness and airsickness sought among patients and friends were, without exception, completely freed of discomfort, provided the drug was taken just before exposure to the motion of the respective means of transportation

In the summer of 1948 an opportunity to use the drug extensively on the U S S America presented itself The surgeon, Dr John E Sheedy, and nursing staff on this vessel cooperated in every detail, and with their assistance sufficient data were collected during four months to warrant a more extensive and intensive study of Dramamine in motion sickness A brief report on the study carried out on the U S S America was submitted to the Chief of Staff and to the Surgeon General of the U S Army The control of seasickness is so important to military operations that complete cooperation for the present study was immediately obtained, and "Operation Seasickness" on which this report is based was planned to begin on November 27, 1948

## 2 PHARMACOLOGY AND TOXICOLOGY OF DRAMAMINE



The pharmacology and toxicology of Dramamine have been examined in the laboratory of the manufacturer, and the data which follow are based on these studies Dramamine is  $\beta$ -dimethylaminoethyl benzohydryl ether 8-chlorotheophyllinate, and contains 54.3 per cent of the ether It is relatively insoluble in water

Antihistaminic potency, measured by the method of Loew, Kaiser and Moore (5), was found to be  $1\frac{1}{2}$  times as great as  $\beta$ -dimethylaminoethyl benzohydryl ether on the basis of the ether content. Spasmodic activity determined by the Magnus technique (6), was found to be less than that of the ether. This is probably due to the insolubility of Dramamine.

Blood pressure and respiration studies were done on cats under pentobarbital anesthesia. In doses of 3-12 mg/Kg, administered intravenously, Dramamine caused a transient fall in blood pressure ranging from 29 to 56 per cent with a return to normal in from 1 to 3 minutes. Respiration was slightly accelerated.

Acute, subacute and chronic toxicity in various animal species were as follows:

a) Acute toxicity: in the mouse the  $LD_{50}$  by the intraperitoneal route was 149 mg/Kg, by mouth 203 mg/Kg. In the rat the  $LD_{50}$  by the oral route was 1,320 mg/Kg.

b) Subacute toxicity: 50 mg to 100 mg/Kg, given daily over a period of 2 to 8 days, caused the death of six cats.

c) Chronic toxicity: large doses were administered orally to eight rats, divided equally between the sexes. The test ran for 63 days with an average daily intake of 22 mg/Kg. There was no weight loss during the experiment. Each animal was weighed every week, and weekly complete blood counts remained normal. After 63 days, half the animals were sacrificed for postmortem examination by decapitation. Administration of the drug to the remaining two males and two females was continued for a total of 119 days, when they, too, were sacrificed. Two normal litters were born to each female before the experiment was terminated. As controls, an equal number of normal rats maintained under identical conditions but untreated with Dramamine were sacrificed at the same time intervals. These animals in the acute, subacute and chronic toxicity experiments showed no gross or microscopic deviation from normal at autopsy.

The data indicated that the oral administration of Dramamine given in doses of 50 to 100 mg every four hours for a period of 6 to 10 days provided an ample margin of safety. However, they provided no information as to the possible frequency of the minor disagreeable side effects produced by other antihistaminic compounds.



### 3 PLAN OF EXPERIMENTAL STUDY

To carry out the study of motion sickness at sea, the following plans were made

a) An Army Transport, the U S A T General Ballou, carrying 1366 replacement troops to Germany was chosen This 13,000 ton ship had been constructed for the U S Navy for use as a freight transport ship on the Pacific It was converted into a troop transport with most compartments below the water level Because of its construction—narrow and high out of the water—a transatlantic passage would be rough, even in the mildest of seas A diagram of the interior arrangements is shown in Figure I

b) The compartment construction of this ship made it possible to assign 500 men in four adjacent sub-level compartments, thus all lived

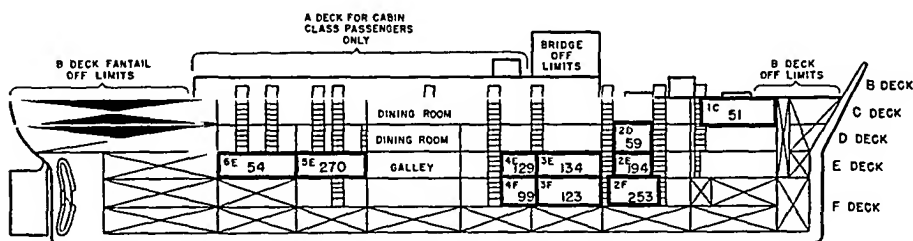


FIG 1

under similar conditions and were subjected to the same motion of the sea

The U S A T General Ballou is a small ship with limited deck space for promenades and with a very small troop lounge Weather conditions prevented the men from enjoying the upper deck Consequently the soldiers who took Dramamine and the placebo were compelled to live in the same confined conditions and were subjected to the same movements of the ship Compartments 3-E, 3-F, 4-E, and 4-F were set aside for the study of 485 men, distributed in these compartments These men were divided into two groups, A and B

*Group A* (prophylactic trial) was divided into two sub-groups Sub-group I, 134 men, occupied compartment 3-E, sub-group II, 123 men, occupied compartment 3-F

These two sub-groups were given the following treatment

Sub-group I When the transport left the harbor of New York, each

of the 134 men in compartment 3-E was given orally a capsule containing 100 mg of Dramamine. The same dose was repeated six hours later at bedtime. For two days after the ship put to sea, a capsule of 100 mg was given to each man before each meal and another on retiring, a total of 400 mg daily.

Sub-group II. When the transport left the harbor of New York, each of the 123 soldiers in compartment 3-F was given a placebo capsule containing lactose identical in appearance to the capsule containing the drug. For two days after the ship put to sea, the same schedule was maintained with the placebo as with Dramamine.

*Group B* (therapeutic trial) was divided into two sub-groups. Sub-group III, 129 men, occupied compartment 4-E, sub-group IV, 99 men, occupied compartment 4-F.

Prophylactic treatment was not given to these two sub-groups. All men were kept under close observation because it was desirable to determine the effectiveness of the drug on individuals after seasickness had developed. It was impossible to anticipate the number of sick individuals. Nevertheless, it was planned to give the drug to all 4-E men who developed motion sickness, and a lactose placebo to all 4-F men who developed motion sickness.

c) A protocol was maintained on every individual who developed seasickness, and daily conferences were held with every seasick person after the vessel left New York and throughout the voyage to Bremerhaven, Germany.

d) Since the voyage required a minimum of ten days, satisfactorily rough weather was anticipated, especially in midwinter. With a variety of rough and smooth weather, varying severity of seasickness was to be expected, especially on a vessel noted for its roll and pitch in a calm sea. Accurate weather conditions were recorded by the ship's officer each day and a comparison of weather with the number of seasick soldiers and with the severity of symptoms was thus facilitated.

e) Each compartment was placed in charge of a non-commissioned officer who was responsible to the ship's Medical Officer. The non-commissioned officer who had no knowledge of the contents of either capsule personally administered the drug or the placebo to his men and observed that each capsule was swallowed. He was also responsible for the attendance of every ill soldier referred to the sick bay. Under

military discipline there is no difficulty in obtaining complete cooperation To insure the cooperation of all soldiers in these four compartments, a sheet of printed instructions was given to each member of the experiment but the substitution of a placebo containing lactose instead of the drug was not mentioned The reason for the administration of the drug was explained in detail, therefore, each soldier knew that the object of the experiment was to prevent or cure seasickness, a malady of which he had some knowledge, even though personal experience with motion sickness might be lacking

f) In addition to the four compartments set aside for intensive study, there were 881 soldiers distributed in six other compartments Opportunity to treat any seasick soldier in this group had been anticipated and since the rumor of the experiment spread throughout the transport, a great number of men reported or were carried to the sick bay for treatment These compartments were 1-C, 51 soldiers, 2-D, 59 soldiers, 2-E, 194 soldiers, 2F, 253 soldiers, 5-E, 270 soldiers, 6-E, 54 soldiers

#### 4 THE EXPERIMENTAL STUDY

The experiment was begun as planned, just as the U S A T General Ballou left New York at 3 30 P M on Saturday, November 27, 1948

Weather conditions were moderately rough for the first five days with a roll of 4 to 15 degrees, and violently rough for the remaining five days with a heavy roll of the vessel from 22 degrees to as high as 35 degrees

Interviews with sick troops were held in the morning between 9 00 A M and 11 30 A M, in the afternoon between 3 00 P M and 5 00 P M and in the evening after 7 00 P M Leading questions were avoided These interviews were recorded on individual protocols by the investigators themselves During the last 48 hours aboard ship 311 soldiers who had been seasick reported for a final interview

#### 5 RESULTS OF THE EXPERIMENTAL STUDY

Each compartment in the experimental groups A and B is reported in detail *Group A—Sub-group I—Compartment 3-E* (Table I-a and b) This group of 134 men continued the prophylactic dose of Dramamine (400 mg daily) for a minimum of 48 hours Of the 134

men who were given Dramamine 100 mg on embarkation and four times daily not one man developed nausea or vomiting while taking

## WEATHER REPORTS

*On Board U S A T General Ballou November 28 to December 6 Inclusive, 1948\**

DATE	TIME	WEATHER	SEA
1948			
11/28	0000	clear	Moderate sea and swell Vessel pitching moderately
	1200	cloudy	Moderate to rough sea and swell Vessel rolling up to 12°
11/29	0000	cloudy	Moderate to rough sea Average swell—Vessel rolling moderately to heavy 15°
	1200	rain	Moderate sea and swell Vessel rolling to 8°
11/30	0000	rain	Moderate swell—Vessel rolling to 5°
	1200	rain	Moderate to rough—Confused swell Vessel rolling to 9°
12/1	0000	cloudy	Moderate—Vessel rolling gently Maximum roll—4°
	1200	cloudy	Rough sea—Heavy swell—Vessel pitching—Rolling heavily up to 28°
12/2	0000	squalls	Rough sea—Heavy swell—Vessel rolling heavily to 22°
	1200	squalls	Very rough sea and heavy swell Vessel rolling up to 35°
12/3	0000	cloudy	Swell heavy—Vessel rolling moderately to heavy up to 19°
	1200	overcast	Moderate to heavy swell—Vessel rolling heavy up to 24°
12/4	0000	squalls	Very rough heavy swell—Vessel rolling moderately to heavy up to 12 to 15°
	1200	rain	Rough sea and heavy swell Vessel rolling 25 to 28°
12/5	0000	rain	Very rough sea—heavy swell
12/6	0000	cloudy	Moderate sea—low swell

\* Temperature recordings, barometric pressure, and wind direction omitted as unimportant

the drug and only two men complained of some dizziness. The 134 men maintained excellent morale, even complained that they were unable to satisfy their appetites. Treatment was then discontinued

entirely on this group for 18 hours, at the end of which time all men who had developed seasickness were advised to report to the sick bay. Forty-one men (30.5 per cent) reported that seasickness had developed 10 to 18 hours after the drug had been omitted. For these men

TABLE I-a  
*Prophylactic Trial*

	COMPARTMENT			
	3 E		3-F	
Number of men	134		123	
Treatment	Dramamine*		X-23 (placebo)	
Hours of treatment	48		48	
Cases of seasickness	No	%	No	%
	2	1.4	35	29**
	(dizziness only)		(nausea, vomiting, dizziness)	

\* Drug dose = 100 mg before each meal and before retiring

\*\* The difference in percentage relief is significant at odds of approximately 1:60

TABLE I-b  
*Therapeutic Trial*

COMPARTMENT 3 E (134 men)			COMPARTMENT 3-F (123 men)		
	No	%		No	%
Number of men who developed seasickness after discontinuing Dramamine	41	31	Number of men who developed seasickness while on X-23	35	29
Dramamine prescribed					
Complete relief after $\frac{1}{2}$ hour	40	98	Complete relief after $\frac{1}{2}$ hour	34	97
Partial relief	1	2	Partial relief	1	3

Dramamine was again prescribed 50 to 100 mg before each meal and on retiring. After 24 hours, one of the 41 men still complained of slight dizziness, but 40 had regained their normal state of health within  $\frac{1}{2}$  to 1 hour after the first dose. Dramamine was administered to these 41 men by the non-commissioned officers every four hours for two days. It was then discontinued. Within twenty-four hours all 41

again reported to the sick bay either because of a second recurrence of severe seasickness, that is nausea and vomiting, or because of inability to retain food or water for at least twelve hours. Dramamine was again administered, and within one hour all but one man were completely relieved. This man continued to complain of some dizziness, but subsequently was able to retain food and water. These 41 men then continued the treatment without recurrence of seasickness before arrival at Bremerhaven.

*Group A—Sub-group II—Compartment 3-F* (Table I-a and b) This group of 123 men continued the placebo according to the schedule for 48 hours. As contrasted with the almost complete absence of seasickness in the group treated with Dramamine, 35 men (28.4 per cent) reported that seasickness had developed within 12 hours after departure from New York harbor. These men, who had severe symptoms, continued the placebo for the two day period, after which time Dramamine was prescribed. They were given 100 mg. of Dramamine which was continued at a dosage level of 400 mg. daily and, with the exception of one man who still complained of dizziness, this group obtained complete relief within one hour after the first dose. Two days later, three men omitted the drug, symptoms returned after six hours, the drug was again prescribed, and each man was relieved within one hour after his first dose. This group of 35 men continued treatment until arrival at Bremerhaven.

*Group B—Sub-group III—Compartment 4-E* (Table II) This group of 129 men remained under observation, but treatment was withheld until severe symptoms had developed. Within 12 hours after departure from New York, 15 (11.6 per cent) reported to the sick bay. Dramamine was prescribed, 100 mg. every five hours and on retiring. Fourteen were immediately relieved of their symptoms and one obtained partial relief. After two days, eight men omitted the treatment, but because of a return of severe symptoms, treatment was again initiated with complete relief of seven and partial relief of one. These 15 men continued treatment and remained free of symptoms until arrival at Bremerhaven.

*Group B—Sub-group IV—Compartment 4-F* (Table II) This group of 99 men was the control group for compartment 4-E. Within 12 hours after departure from New York, 33 (33.3 per cent) reported to the

sick bay These men were given a placebo, one capsule every five hours and upon retiring Two days later, they again reported to the sick bay and the following facts were recorded nineteen men whose complaints had been nausea and dizziness had recovered within 12 hours The lactose treatment of the 19 men was discontinued, and they had no return of symptoms during the remaining days of the voyage Fourteen men became progressively worse on the placebo and now complained of excessive nausea, extreme dizziness and prolonged vomiting The 14 men whose symptoms had become pro-

TABLE II  
*Therapeutic Trial*

Compartment Number of men Treatment	4-E 129 Dramamine*		4-F 99 X 23 (placebo)	
	No	%	No	%
Cases of seasickness within 12 hours after departure	15	12	33	33
Complete relief within $\frac{1}{2}$ hour	14	93**	19	58**
No relief	1	7	14	42
			Dramamine prescribed to above 14 men and complete relief ob- tained in $\frac{1}{2}$ hour	

\* Drug dose = 100 mg before each meal and before retiring

\*\* The difference in percentage relief is significant at odds of approximately 1 60

gressively worse on the placebo were then given capsules of Dramamine according to the schedule (every five hours and upon retiring) Complete relief followed in all 14 within one-half hour after the first dose of 100 mg After two days three men discontinued the drug, symptoms returned, and again the drug was prescribed These men again completely recovered one-half hour after the first dose The 14 men then continued the treatment and remained free of symptoms until the completion of the voyage to Bremerhaven

Among the men in compartments 1-C, 2-D, 2-E, 2-F, 5-E and 6-E, none of whom received prophylactic treatment, there were many

instances of seasickness, and these men were treated with the drug and with a placebo (Table III-a) They were followed with the same care as were the men in the experimental groups A and B Valuable

TABLE III-a  
*Therapeutic Trial*

Combined compartments	1-C, 2 D, 2 E, 2-F, 5-E and 6-E	
Number of men	881	
	No	%
Cases of seasickness	195	22
Dramamine		
Complete relief	187	96
No relief	8	4
Treatment discontinued after 2 days	187	
Relapses	44	24

TABLE III-b  
*Treatment of Relapses*

Number of men	44	
	No	%
Group a—Dramamine	18	
Complete relief	15*	83**
No relief	3	17
Group b—X-23 (placebo)	26	
Complete relief	2	8**
No relief	24	92
Group b—Dramamine—subsequent treatment	24	
Complete relief	16*	
Unreported†	8	

\* Complete relief was obtained in every instance within one hour after the first capsules of Dramamine (50 to 100 mg)

\*\* The difference in percentage relief is significant at odds of approximately 1 10<sup>6</sup>

† Eight men failed to report at time of final conference

information, which supplemented that of the experimental groups, was obtained

The number of occupants in these six compartments totaled 881 During the voyage 195 (22.1 per cent) of these men reported severe



symptoms of seasickness Each one immediately began treatment with Dramamine, 100 mg every five hours and on retiring Of this group of 195 men, 187 were completely relieved within one hour after the first capsule, but 8 derived no benefit from the 400 mg admin-

TABLE IV  
*Summary of Therapeutic Use of Dramamine*

Total number of men on board—U S A T General Ballou	1366	
Total number of cases of seasickness	319	
Total number of cases of seasickness treated with Dramamine and recorded	300	
Complete relief obtained	288	96 0%
Partial relief or failure	12	4 0%
Total number of relapses induced by omission of Dramamine	89	
After Dramamine complete relief obtained	84	94 4%
After Dramamine partial relief or failure	5	5 6%
Total number of cases treated with Dramamine including relapses	389	
Complete relief obtained	372	95 6%
Partial relief or failure	17	4 4%

TABLE V  
*Number and Percentage of Failures of Response to Dramamine Compared with Similar Failure with Placebo*

	DRAMAMINE			X-23 (PLACEBO)		
	No treated	No failures	% failures	No treated	No failures	% failures
Prophylactic use	134	2	1 4*	123	35	28 4*
Therapeutic use	319	8	2 5*	59	38	64 4*

\* The difference in percentage protection or relief between the Dramamine and placebo group is significant at odds of approximately 1 10<sup>6</sup>

istered daily After two days of treatment the drug was discontinued in these 187 men and 44 of this group then developed nausea and vomiting within twelve hours

In order that additional confirmatory information concerning the value of the drug could be gathered, the above group of 44 was divided

and treated as follows 18 men were given Dramamine, 400 mg daily (Table III-b) Within one hour after the first dose, symptoms were completely relieved in 15, but there was no improvement in 3 Twenty-six were given a placebo, a capsule every five hours and on retiring, after 24 hours these men were interviewed and 2 had regained their normal condition but 24 were unimproved or worse These men complained that the capsules (which they did not know contained lactose) had made them much worse, therefore, these 24 men were then given Dramamine and were advised to report on their condition the following day Sixteen appeared at the sick bay Of this number, only one man failed to be relieved within one hour after the first capsule of 100 mg It was impossible to obtain information from 8 men of the group of 24 because of the termination of the voyage

## 6 DISCUSSION

Seasickness is an acute illness which may clear spontaneously within two or three days after onset when the apparatus of equilibrium accustoms itself to the motion of a ship at sea The serious manifestation is the chronic state, characterized by nausea, dizziness and vomiting of such violence that dehydration followed by acidosis occurs Travelers on commercial liners have no conception of the seriousness of the condition, as the "afflicted" remain in their cabins, attended by stewards, or they lie prostrate on deckchairs With nursing and encouragement to take fluids serious complications are usually avoided No such service is available on a troop ship, and only those who have accompanied troops at sea can realize the administrative problem

A brief description of the voyage to Bremerhaven will enlighten those who are not aware of the complications Within twelve hours after the U S A T General Ballou left New York harbor, the corridors of compartments were congested by sick men, so ill that they were unable to reach the latrines The men who reached these areas were unable to return to their compartments and remained stretched out in semi-conscious condition on the floors until more seaworthy individuals managed to drag them to the sick bay or back to their hammocks The latrines became temporarily indescribably repulsive According to the ship's surgeon, such an overwhelming sickness would have

filled the forty available beds in the sick bay and then, because of inadequate help, dehydration would have followed. On the previous trip of the U S A T General Ballou from Bremerhaven, more than 100 intravenous injections of saline solution were required to relieve a number of dehydrated persons. This necessitated continuous work, night and day, throughout the trip and consequently it was necessary to neglect other important duties.

In order to prevent a repetition of similar disorder, all seasick soldiers were given Dramamine as soon as they reached the sick bay and treatment was continued according to the schedule previously outlined. During the ten days at sea, the administration of Dramamine successfully controlled all severe symptoms in every seasick soldier with the exception of 8 men. No soldier required hospital admission or intravenous treatment.

When the control subjects developed seasickness, many were brought to the sick bay in a semi-conscious state. A number had lain in their hammocks for three days without food or drink and consequently could not walk or even stand when they were brought to the surgeon's office. Many lay stretched out on the floor, too ill to retain even a capsule of the drug. To overcome this difficult situation, the drug was administered rectally. The capsule was punctured at each end, inserted into the rectum, and 30 cc of salt solution was injected into the rectum. With rectal, as well as oral administration, relief was prompt. Within one-half hour, the sick soldier had improved to such an extent that he was aware of his surroundings, soon he asked for fluids and within an hour he was able to retain both fluids and solid food. These dramatic examples occurred repeatedly during the 10 days of this experimental study, and gave information as to what might be accomplished by rectal administration of the drug, when the patient was unable to retain oral medication.

The action of Dramamine on the prevention and control of motion sickness is as yet not definitely established. Studies are in progress to attempt to explain the mechanism.

Throughout the duration of this experiment, careful watch was maintained for unpleasant symptoms from the drug but in no instance, even though thousands of capsules were administered to more than 300 men, was there a complaint or evidence of discomfort which neces-

sitated discontinuance of treatment. This observation differs from the reaction to other antihistaminic drugs, which, when used for the control of allergic manifestations, cause unpleasant symptoms in 25 to 60 per cent of patients. The usual amount of Dramamine required to prevent symptoms was 400 mg, in 24 hours, in a few instances, as much as 800 mg of the drug was necessary.

It was of interest to note how many of the men who developed seasickness on their voyage to Bremerhaven had previously been at sea. Of 608 men questioned, only 78 had been to sea on one or more former occasions. Forty of these had experienced seasickness on their earlier trips.

## 7 CONCLUSIONS

The accumulated data indicate that Dramamine is a powerful, non-toxic, prophylactic and therapeutic drug, which can be used to control the symptoms of seasickness. The drug may be taken orally or it may be administered rectally, and no untoward side effects have been noted, using the standard dose of 400 mg. In a group of more than 300 men, who developed moderate to violent symptoms of seasickness while en route to Bremerhaven, complete relief was obtained in all but 11 cases.

## SUMMARY

1 A study of seasickness was planned and executed on the U S A T General Ballou.

2 This transport carried 1366 soldiers to Bremerhaven, Germany. The voyage began on November 27, 1948 and, after a rough passage, terminated on December 7, 1948. Complete cooperation was given by the Surgeon General's office and by the Transport Command.

3 Four compartments on the transport were set aside for the controlled study of the 485 men assigned to these compartments and subjected to the same motion of the sea.

4 Treatment was planned so that half of the men were given Dramamine or a placebo at the time of departure from New York harbor, the other half were given Dramamine or a placebo 2 to 12 hours after the onset of symptoms of seasickness. Adequate control groups were given a placebo. The dose of Dramamine was 100 mg every five hours and before retiring.

5 Dramamine prevented seasickness in all but 2 men of 134 who occupied compartment 3-E, the placebo failed to relieve the symptoms in all controls who developed true seasickness in compartment 3-F. However, this control group (34 men) obtained complete relief of symptoms within one hour after the first dose of Dramamine was administered.

The drug gave complete relief to 14 men in compartment 4-E who developed symptoms three or more hours after the transport left New York. A placebo failed to relieve 14 men in compartment 4-F but these men obtained complete relief one-half hour after Dramamine

TABLE VI  
*Return Trip of the U S A T General Ballou from Bremerhaven*

Total number of seasick passengers	109	
	No	%
Dramamine prescribed	73	100
Complete relief	65	89
24 hours	56	
48 hours	9	
Partial relief	5	7
No relief	3	4
Placebos prescribed	36	
Complete relief (24 hrs)	16	44
No relief	20	56
Dramamine prescribed	20	
Complete relief	14	70
Partial relief	6	30

was substituted for the placebo. Nineteen men who developed symptoms three or more hours after the transport left New York recovered while on a placebo. These men required no medication during the last seven days of the voyage to Bremerhaven.

6 Among 881 men who occupied other compartments on the transport, 195 cases of severe seasickness developed. Of these, 187 derived complete relief one-half hour after the administration of Dramamine.

7 Relapses were induced by the substitution of a placebo, but these symptoms were relieved within one-half hour after the administration of Dramamine (Table IV).

8 During a period of 10 days, Dramamine was given to 389 cases of seasickness. Of this number, 372 were completely relieved of symp-

toms within one hour after the first dose of 100 mg Seventeen cases derived only partial or no relief (Table V)

9 A dose of 100 mg prescribed every five hours and before retiring was adequate to control the most distressing symptoms When the patient was unable to retain a capsule administered orally, he did retain and absorb the drug given rectally The benefit derived by this route of administration was as rapid and as complete as that derived by the oral route

10 No reaction to Dramamine was encountered by any soldier to whom it was administered during the period of ten days

#### SUPPLEMENTARY NOTE

On the return trip of the General Ballou from Bremerhaven in December, 1948, Lt J L Soelling continued the experimental study of seasickness The following table (Table VI) is a record of the seasick passengers The sex of the majority of these seasick passengers is female Dramamine was prescribed to 73 passengers 56 (77 per cent) were completely relieved in 24 hours, 9 (12 per cent) were completely relieved in 48 hours (a total of 89 per cent), 5 (7 per cent) were partially relieved, and 3 (4 per cent) of the group obtained no relief To 36 seasick individuals, he gave a placebo Sixteen (44 per cent) of this number recovered normal health after 24 hours, but 20 (56 per cent) of the group were worse than before the placebo had been prescribed He then prescribed Dramamine to the 20 sick passengers Fourteen (70 per cent) derived relief within one hour and 6 (30 per cent) derived partial relief in one hour

The results of this supplementary report parallel those of the preliminary experiments

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# MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

HURD HALL, THE JOHNS HOPKINS HOSPITAL,

MONDAY, FEBRUARY 14, 1949

*Dr Harvey* The first paper on the program tonight is

## MOTION SICKNESS—PREVENTION AND TREATMENT

by Drs Leslie N Gay and Paul E Carliner (This paper is published in full on page 470 of this issue of the Bulletin)

*Dr Harvey* Dr Gay and Dr Carliner's paper is open for discussion *Dr Bard*

*Dr Bard* I am a little sick of seasickness myself I would like to make one or two comments *Dr Harvey* My contact with seasickness was during the war, as a member of an N R C committee which was asked to find some information about it and, as Dr Gay pointed out, the most effective preventive turned out to be hyoscine

From a military point of view, prevention over relatively short periods of time in airborne and amphibious operations was the main problem, so tests were confined to relatively short periods of time—up to, let us say, twelve hours

One thing that came from these studies was the great importance of methodology One had always to take great care not to be fooled Those of us who started in 1942 were often fooled and one of the chief sources of trouble was the statistical one, the question of whether, in a group of unselected personnel—about which you knew absolutely nothing of their past histories—you had within the group a sufficient number to provide the entire spectrum of susceptibility Unless the percentage sick in the control group was pretty high, you have to have very large numbers, as our statistical studies showed

As far as methodology was concerned, Dr Gay referred to the matter of position, which is extremely important He referred to Dr Tyler's results, and I could refer him to others, which showed that as far as position of head is concerned one can reduce the incidence from somewhat in the neighborhood of 68 per cent to zero, simply by changing the angle of the head in relation to the motion of a swing by about forty degrees So one wants to know whether these men of Dr Gay's moved around, whether the treated men changed their positions more often than the untreated, who, for the most part, stayed flat on their backs, or flat on their stomachs, because they did not have the benefit of the treatment And then one wonders whether that change in position added to the apparent effect of the drug

It seems to me that the great contribution here is one of treatment of people already sick I know of no evidence heretofore put forward which indicates that you could get anything like this result by giving the drug to very sick men But I would like to point out that an incidence of 28 or 29 per cent in controls is not a high incidence Hyoscine would certainly, on the basis of Dr Tyler's work, have

protected nearly as many men as this drug, with the control incidence of only 28 per cent To me, the outstanding feature of this work is the very marked effect on already sick men

*Dr Harvey* Dr Tyler, I didn't see you there Would you like to make any comments?

*Dr Tyler* Well, I can appreciate fully the trials and tribulations that Dr Gay must have gone through in running the experiment, particularly an experiment on an Army transport

The results that have been presented here tonight are extremely interesting to me In the first place, the indication that a medication other than hyoscine is effective in preventing motion sickness is indeed a real contribution One thing I would surely like to see would be more tests, with a range of seasickness in the controls up to, say, 70 per cent When you get an incidence of around 70 per cent you have adequate experimental conditions to assay the effectiveness of a proposed remedy

There are one or two items that come into my mind that have no particular bearing on Dr Gay's work I am amazed by the Army's, and particularly the Surgeon General's, ignorance of some of the things that have been accomplished in regard to the problem of motion sickness In the first place, there is the question of the side effects of hyoscine

Now the administration of hyoscine in a dosage that is effective in preventing motion sickness—say up to six-tenths of a milligram—is surprisingly without any serious side effects on the combat efficiency of men as determined by a wide variety of tests In fact, the Army could find in reports to the Surgeon General from the C M R that they have a very effective remedy that will prevent motion sickness in about 80 per cent of susceptibles when the incidence in the controls is 23 per cent With an incidence of 60 per cent, one-half is protected In addition to preventing motion sickness, a remedy available to the Army since 1938, one that contains hyoscine, also significantly improves the marksmanship of the average enlisted man

I would like to emphasize what Dr Bard pointed out here We have never been able to find any remedy—barbiturates or hyoscine, or a combination of hyoscine and barbiturates—that could be used therapeutically (as distinct from prophylactically), and apparently we have such an agent now That is indeed a real contribution to the problem

*Dr Harvey* Is there any further discussion, or any questions anyone would like to ask?

*Dr Gay* I would like to make a few remarks at this time, if you don't mind before we leave the subject

In the first place, of course, I don't like to argue with Dr Tyler and Dr Bard, as they are physiologists, but if there are any remedies that can be used for the treatment of seasickness they haven't appeared in the literature, to my knowledge, since 1942 Now, if I can take just a moment to point out some of the comments that were made by the men who were assigned to do seasickness studies in Eng-



land, Dr Holling and his co-workers found that while hyoscine was effective, certainly they didn't recommend it for repeated doses in the control of seasickness. In their experiments they definitely said that "Chronic sufferers from seasickness"—I am quoting them—"may be astonished to learn that on most days through the year an obstinate and baffling calm haunts the waters around England." They commented that the waters were so calm in all of their studies they were not really able to try out their drugs in a very effective manner.

The same thing appeared in Dr Best's article in which he, himself, said that attempts to make seasickness trials, using large numbers of subjects, were ruined by fair weather, and he said it is unlikely that a carefully controlled study at sea will be possible under peacetime conditions.

We feel that we have carried out a study under peacetime conditions and that it was very well controlled. Now, if we continue this study, and if we completely control seasickness so that no man on board ship, say 1,300 men, have seasickness, I can't see how statistics can be of much avail to say that the drug is not a good therapeutic agent from that point of view. We have had very few failures that we can say were real failures in the 311 men that were treated.

*Dr Harvey:* Thank you very much, Dr Gay.

The final paper tonight is on

#### TRANSFORMATION ON PNEUMOCOCCAL TYPES A REVIEW

by Dr Maclyn McCarty, of the Rockefeller Institute. Dr McCarty will present a review of the work which has been done by himself and his associates at the Rockefeller Institute. (A similar review was published by Dr McCarty in *Bacteriological Reviews*) 1946, 10, 63.

It gives me great pleasure to introduce Dr McCarty to you. He is a graduate from this School in 1937, and after three years of house service he went for a year's fellowship with Dr Avery of New York. Since that time he has been at the Rockefeller Institute. Dr McCarty.

*Dr Harvey:* Dr McCarty's paper is now open for discussion. Dr Turner, do you have any comments to make?

*Dr Turner:* Dr Harvey, I am not qualified to discuss in detail Dr McCarty's work, but I would like to point out some of the implications of the work which I think are very important indeed.

From the standpoint of the epidemiologist, we really do not know what causes an epidemic—whether it is a change in the host, or in the infecting organism. The preponderance of evidence, I might say, seems to be on the side of the change in the host, that is, herd immunity is built up and when that wanes epidemics occur. Yet that fails to explain striking seasonal correlations, so that the other theory assumes that a transformation of the parasite occurs.

Dr McCarty's work has been done largely *in vitro*, but one wonders how often the same phenomenon may occur in nature.

We have one other striking example of a similar phenomenon the Shope fibroma virus which produces a benign disease in rabbits, and the myxoma virus, which produces a very virulent disease. Combining heat inactivated myxoma virus with fibroma virus will impart to the latter the characteristics of the myxoma virus.

Another practical implication concerns the stability of immunological types. Are they as stable as we think?

Thirdly—I am taking off into thin air now—one of the current theories about the causation of cancer is the so-called transformation of the somatic cell. While work on the pneumococcus may be a long way removed from somatic cell mutation, the more we know about what causes cells to change their characteristics, perhaps the further along we shall be on that problem too.

*Dr Harvey:* Are there any further questions or discussion of Dr McCarty's paper?

Then the meeting is closed.

## BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

*Blood Clotting and Allied Problems* Transactions of the First Conference, Josiah Macy, Jr Foundation

This publication is essentially a transcription of the informal discussions which took place at a conference sponsored by the Josiah Macy, Jr Foundation in February 1948. The participants included many of the best known investigators in the field of blood clotting, representing several branches of medical science. Some of the recent developments in coagulation research are discussed in detail. Much space is devoted to description and discussion of various methods for determination of prothrombin. The authors are unable to agree upon a procedure for standardizing prothrombin assays although the need for such standardization is emphasized.

Those interested in blood coagulation should not fail to read this little volume. However, it is not in any sense a review of the subject, and the random manner of presentation will make reading difficult for those without particular interest in this field. The heated debate and vituperation which characterize some of the discussion add space to what is otherwise a very technical presentation.

C L C

*Blood Transfusion* By ELMER L. DEGOWIN, ROBERT C. HARDIN, and JOHN B. ALSEVER. W. B. Saunders Company, Philadelphia & London, 1949. 587 pages.

This is an attractively published little book, clearly and simply written. It is apparently designed as a fairly complete manual for laboratory technicians in blood banks and suits its purpose admirably. There are a few simple line drawings where required and no padding with unnecessary illustrations. The historical and scientific background of the various aspects of blood banking is simply and clearly given. The laboratory procedures themselves are given in great detail. This is by all odds the best conceived and most intelligently organized book which has yet been produced on the subject.

M M R

*Human Biochemistry* By ISRAEL S. KLEINER. 2nd ed. C. V. Mosby Co., St. Louis, 1948.

Israel Kleiner, professor of biochemistry at the New York Medical College, in the preface to the first edition of his book, mentions the vital part played by biochemistry in medicine and its increasingly important role in diagnosis and therapeutics. Stated in this mild way, the author reflects the gradually enlarging appreciation of such values on the part of medical investigators, clinicians, and

biological scientists in many fields. He falls short of presenting the ringing challenge and needed dominance of such fundamental science in medicine and biology of the immediate future.

In the second edition, as in the first, the author attempts to present biochemistry in a more available form by not giving or discussing much of the evidence for the quite definitely stated material, and at the same time he attempts to include a large proportion of the subject-matter of biochemistry and some of its relation to medicine. The result to the reviewer's mind provides certain characteristics about the book which help to define its sphere of usefulness. These are that the contents form a fairly convenient reference for preliminary orientation on any subject, they also suggest some of the many relationships of biochemical conclusions to other fields. A person desiring a quick survey of a subject might turn to this book with advantage. He should do so, however, only with the realization that the brief statements given can be misleading (such as the effect of glutamic acid on brain function, page 339) and that a satisfactory understanding of the given subject can be acquired only by a study of the underlying evidence, for this he will have to look elsewhere. Professor Kleiner has not included many references, but those given should help to orient the student.

One interesting feature is the reference to clinical applications in a number of instances. However, these are relatively restricted in both number and scope, and the author does not attempt to correlate and unify the biochemical aspects of various disease states.

It should be realized that it is an almost impossible task to write a text-book in the fields of biochemistry and medicine that covers the ground and yet includes an adequate discussion of the evidence available on every subject, unless, perhaps, a new approach is made to the whole field. Professor Kleiner purposely has sacrificed a marshalling of the evidence and a more narrative style to put down as many of the conclusions on these subjects in as reasonable a space as he could. Subject-matter includes a good deal of recent work and the style is clear and readily followed.

F W B Jr

*Surgical Treatment of the Abdomen* By F W BANCROFT, AND P A WADE  
J B Lippincott Company, 1947 460 Illustrations, \$15 00

This is an addition to the series of books on surgical treatment under editorial revision by the senior author. Abdominal diseases are discussed by thirty-six authors contributing twenty five chapters. This contributes to a lack of cohesion and an integrated point of view. Some chapters are written at the surgeon's level, others at the intern's or medical student's level. Of 990 pages, less than two-thirds are devoted strictly to abdominal surgery. The remaining third represents separate sections on anesthesia, diet, ward care and operating room behavior. Much of this additional material is covered in other texts or under other headings elsewhere in this book. One such extraneous chapter, however, is

a classic By Mont Reid and Jean Stevenson, it discusses in peerless fashion details of surgical technique and the surgeon's conduct and the general philosophy of operating room treatment This essay should be required yearly reading for every surgeon and aspirant On a subject that could be platitudinous and pedestrian, it is fresh, glowing, inspiring From this professional and literary height the sections vary all the way down to a treatise on diet that ascribes to intestinal putrefaction the blame for a large share of abdominal disease

W E G

## BOOKS RECEIVED FOR REVIEW

- Acclimatization in the Andes* By CARLOS MONGE 130 pp , \$2 75 *Published by The Johns Hopkins Press*
- Blood Transfusion* By ELMER L. DEGOWIN 587 pp , 200 drawings, \$9 00  
*Published by W B Saunders Co , Philadelphia, Pa*
- Clinical Aspects and Treatment of Surgical Infections* By FRANK L. MELENEY  
840 pp , 287 figures, \$12 00 *Published by W B Saunders Co , Philadelphia, Pa*
- 1948 Facts about Nursing* 106 pp , 35 cents *Published by American Nurses' Association, 1790 Broadway, New York*
- Mayo Clinic Diet Manual* By the Committee on Dietetics of the Mayo Clinic,  
329 pp , \$4 00 *Published by W B Saunders Co , Philadelphia, Pa*
- Tests and Measurements Applied to Nursing Education* By HYMAN KRAKOWER,  
PHD 176 pp , \$3 50 *Published by G P Putnam's Sons, New York City*
- The Business Side of Medical Practice* 2nd Edition By THEODORE WIPRUD  
232 pp , 22 figures *Published by W B Saunders Co , Phila , Pa \$3 50*
- Your Child or Mine The Story of the Cerebral-Palsied Child* By MARY LOUISE  
HART BURTON in collaboration with SAGE HOLTER JENNINGS 64 pp \$1 25  
*Published by Coward-McCann, Inc New York City*



# A FACTOR IN OLD HEPATITIS SERUM CAPABLE OF AGGLUTINATING CHICKEN RED CELLS\*

FREDERIK B BANG

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With the technical assistance of MISS MARIE A FOARD

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During the course of a study of the agglutination of red cells previously treated with Newcastle Virus (1), it was discovered that certain hepatitis sera in a very high dilution were capable of agglutinating normal washed red blood cells from certain chickens. Since these sera were often acute phase sera† the question arose whether the agglutinating factor might be the virus of infectious hepatitis. Although our results indicate that the agglutinating factor is not the virus, the nature of the problem makes it impossible to state this with certainty. It was further demonstrated that the reaction of agglutination or pattern formation could be produced with red cells from only certain chickens, and that the degree of reaction could be partially influenced by the method of washing the glassware. Fresh hepatitis serum did not agglutinate these red cells. Cells exposed to this factor did not become resistant to several of the viruses capable of agglutinating chicken red cells.

## METHOD

Tests for agglutination were performed by making serial twofold dilutions of the serum in a one per cent phosphate buffered saline of 14 molarity (pH 7.2). Dilutions were made in Wassermann tubes and the end point judged by the pattern which formed at the bottom of the tube as the red cells settled in 45–60 minutes at room temperature (20–25°C). Several tests run simultaneously in the icebox, incubator, and at room temperature showed no difference. Routinely, all glassware was first cleaned mechanically by rubbing with a test tube brush.

\* Supported in part by a grant in aid from the Nat. Inst. of Health of the USPHS and conducted in part under contract with the Office of Naval Research, U. S. Navy.

† Sera taken during the acute phase of the hepatitis.



without soap, then terminally cleaned by immersion in dichromate cleaning solution for 24 hours. It was finally rinsed five times in tap water and twice in distilled water. Chicken red cells were obtained by washing citrated blood with one per cent phosphate buffered saline (pH 7.2) two times. A final concentration of about one per cent of packed cells was used. No red cell preparations older than a week were used. The old sera studied here were serial bleedings obtained by Dr. George S. Mirick from cases of acute hepatitis in Guam and at Rockefeller Institute, New York (1944-1945). They had been stored at about 4°C since then. Only clear serum was tested. Cultures for aerobic and anaerobic organisms and fungi were done on 29 negative agglutinators, five of which were positive, and on 8 positive agglutinators, 4 of which were positive.

The first serum found capable of agglutinating normal chicken red blood cells was highly specific in that only about one half of the preparations of red cells obtained from different chickens were capable of any agglutination by this serum. Red cells obtained from the positive chickens were repeatedly and uniformly agglutinated by that particular serum in dilutions of 1/5000 or more. It was subsequently found that when a chicken furnished red cells capable of agglutination by one positive serum, its red cells would usually be agglutinated by any other positive serum (Table I). That this specificity of cells and serum had however nothing to do with blood types in man is likely because different sera from the same individual patient failed to agglutinate the same red cells. This specificity is reminiscent of that described for vaccinia and ectromelia hemagglutination (2), and in the light of the known individual differences in chicken blood (3) is not surprising.

Following this preliminary characterization of the reaction, the study was enlarged and Dr. Mirick undertook to check the ability of a number of other hepatitis sera to agglutinate these red cells. He obtained positive results with sera which in our hands had been negative with the same red cell-serum combination. It was then discovered that the type of cleaning and washing to which the glassware had been subjected was the cause of the difference in results and that a serum which was negative in tubes previously washed with cleaning solution was positive in tubes washed in surgical green soap before

rinsing That the effect of the soap was a potentiating one and very persistent may be seen by the large number of times that it was necessary to rinse the tubes to get rid of this effect

Not only was the type of washing capable of making certain negative sera positive but sera which were capable in a dilution of 1/800 of

TABLE I

*Agglutination of Washed Chicken Red Cells by Old Sera in Dilution of 1/100 or More*

CELLS FROM CHICKEN %	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
63		0		0	0										
53		0			0										
134		0													
168						0									
190	0					0									
11		0			0										
26															
76		0		0	0										
5		0		0	0										
195		0													
165		0													
105	+		+			+									
174	+					+									
138	0	+													
125		+													
122	+	+													
119		0	+												
112	+	+													
92		+			0	+	0	0	0	0	0	0	+	0	0
31				+											
26		+		+											
8		+		+	0	0	0	0	0	0	0	0	0	0	0
3		+		+	0	0									
64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

agglutinating red cells were made more powerful by titration in soap-washed tubes (Table III)

Attempts were, therefore, made to run many of the tests in both soap-washed and acid washed tubes This established the potentiating effect of the previous treatment with soap, but the difficulty of exactly reproducing the amount of soap left behind in each tube led us to add graded amounts of soap to the different tubes In this way it was

found that a 1/80,000 dilution of a concentrated suspension of green surgical soap potentiated the ability of certain sera to agglutinate chicken red cells (Table IV) Higher concentrations either agglutinated all cells or hemolyzed them

TABLE II

*Effect of Type of Washing and Number of Rinsings on the Red Cell Agglutination Produced by Serum Negative in Acid Cleaned Tubes*

	NUMBER OF RINSINGS		RESULTS									
	Tap water	Dis tilled water	Dilution of serum									
			1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12800	1/25600	1/51200
Soap cleaned	2	0	0	+++	+++	+++	+++	+++	++	+	0	0
	5	2	+++	+++	+++	+++	+++	++	0	0	0	0
	10	10	0	0	0	0	0	0	0	0	0	0
Acid cleaned	2	0	0	0	0	0	0	0	0	0	0	0
	5	2	0	0	0	0	0	0	0	0	0	0
	10	10	0	0	0	0	0	0	0	0	0	0

TABLE III

*Potentiating Effect of Soap Washed Tubes on Agglutination by a Serum Positive in Acid Cleaned Tubes*

	NUMBER OF RINSINGS		DILUTION OF SERUM									
	Tap water	Dis tilled water	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12800	1/25600	1/51200
Soap cleaned	2	0	+++	+++	+++	+++	+++	+++	+++	+++	++	++
	5	2	+++	+++	+++	+++	+++	+++	++	++	+	+
	10	10	+++	+++	+++	+++	+++	++	0	++	0	0
Acid cleaned	2	0	+++	+++	+++	+++	++	+	0	0	0	0
	5	2	+++	+++	+++	+++	++	+	0	0	0	0
	10	10	+++	+++	+++	+++	++	+	0	0	0	0

It is interesting to note in this table that the presence of soap did not make cells ( $\approx 5$ ) which were not ordinarily agglutinated by positive sera, capable of agglutination This would indicate a degree of specificity of effect It was, however, true that larger concentrations of soap were capable in themselves of agglutinating normal chicken

red cells, and because of this and the difficulty of controlling the reaction, we continued our studies using only tubes cleaned in acid and routinely washed three times in tap water and twice in distilled water. All results reported subsequently in this paper are then from such tubes.

In a limited number of experiments we attempted to partially characterize the factor in the serum which is capable of agglutinating the chicken red cells. For this only strongly positive sera were used.

TABLE IV

*Effect of Minute Amounts of Soap Added to the Serum Before the Addition of Red Cells*

RED CELLS FROM CHICKEN, %	AMOUNT OF SOAP FINAL CONC	SERUM DILUTION									
		1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12800	1/25600
92	1/80,000	0	+	++	++	++	++	0	0	0	0
	1/160,000	0	+	+	++	++	0	0	0	0	0
	None	0	0	0	0	0	0	0	0	0	0
8	1/80,000	+	++	+++	+++	+++	+++	++	+	0	0
	1/160,000	0	0	++	+++	+++	+	0	0	0	0
	None	0	+	+	++	+	+	++	0	0	0
5	1/80,000	0	0	0	0	0	0	0	0	0	0
	1/160,000	0	0	0	0	0	0	0	0	0	0
	None	0	0	0	0	0	0	0	0	0	0

*Size of particle or molecules responsible for agglutination*—Assuming a density greater than water, larger molecules should be sedimented in a centrifugal field. If the agglutination here studied was caused by a virus of the usual density it might be expected that a force of approximately 50-90,000 g for one hour in a physiological salt solution would produce a change in the distribution of particles of a size of 50  $\mu$  or larger.

In two tests run in a Sorval angle centrifuge at about 13,000 r p m (17,000 g) and one test in the air-driven ultracentrifuge at the Rockefeller Institute for Medical Research at Princeton, N J at 24,000 r p m (50-90,000 g) there was no evidence of sedimentation. This latter centrifuge has been repeatedly used for the sedimentation of equine encephalomyelitis which is about 50  $\mu$  in size (4, 5).

## METHOD

Serum was diluted 1/10 in normal saline and the centrifugation carried out in a pre-cooled head (4°C). Two separate sera were tested, one with a titer of 1 3200, the other 1 200. After centrifugation for one hour the top one cc was taken carefully off, then a sample from the middle, and finally the bottom fluid was mixed with the film which had collected at the bottom of the tube. In no case was the titer of the top sample after centrifugation below that of the original fluid, and the titer of the pellet resuspended in the fluid at the bottom at no time indicated any increase of red cell agglutinating activity.

These results indicate that the factor or particle causing the agglutination is less than 50 m $\mu$  but in the absence of data on the density of this component no further study was practicable in this laboratory.

On the other hand, in three separate experiments, dialysis through a viscose collodion sac of two positive sera failed to demonstrate the passage of any of the red cell agglutinating factor through the pores of the collodion sac. Again care was taken to use sera with a high titer (1/600–1/3200) and this was diluted 1/10 in saline before dialysis. This might indicate that its "molecular weight" is above 6,000, since trypsin inhibitor, which has a molecular weight of about 6,000, will diffuse through such membranes (6).

Crude fractionation of the serum by dilution in distilled water showed that the agglutinating factor could be precipitated and recovered on resuspension in saline, but that the greater fraction was left behind in the supernatant (Table V). Precipitation of serum with both 50 per cent and 75 per cent ammonium sulphate brought about a concentration of the factor in the precipitate in both instances.

*Relation to Hepatitis*—The great need for a practical test for the presence of the virus of infectious hepatitis in serum led us to inquire carefully into the possibility that the reaction here studied was caused by that virus.

1 Table VI shows that positive sera were found throughout the course of infectious hepatitis and that high titers were obtained after the acute disease was over. Positive reactions were more frequent early in the course of the illness. The positive results obtained in late convalescence would not in themselves rule out a viral etiology of the phenomenon (7).

2 A comparison of the "neutralizing ability" of acute serum with serum taken one to two months later in 6 cases of infectious hepatitis has failed to show any change in the "neutralization" of 10 hemagglutinating doses of the agent. This would tend to exclude the virus

TABLE V

*Precipitation of Agglutinating Factor From Sera on Dilution in Distilled Water (Red Cell Tests done in Saline)*

DIL IN WATER	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240	1/20480	1/40960
1/2 Precip Supernat	+++ h	+++ +++	+++ +++	+++ +++	+++ +++	+++ ++	+	+	0	0		
1/5 Precip Supernat	h h	+++ h	+++ +++	+++ +++	+++ +++	+++ +++	++ +++	+	0	0		
1/10 Precip Supernat	+++ h	+++ h	+++ +++	+++ +++	+++ +++	0 +++	0 +++	0 ++	0 0	0 0	0 0	0 0
1/10 Precip Supernat		+++ h	+++ h	+++ +++	+++ +++	+	0 +++	0 ++	0 +	0 0	0 0	0 0
1/20 Precip Supernat	+++	+++	+++ h	+++ +++	+	0	0	0	0	0	0	0

Note Dilution of Precipitate above is on the basis of original volume of serum although the small volume of precipitate probably was less than 1/50 of the original volume

TABLE VI

*Relation of Day of Disease to Subsequent Hemagglutination*

DAY OF ILLNESS	% TESTED	% POSITIVE	%
0-15	19	8	42.1
16-30	27	2	7.4
30-45	16	2	12.5
36+	16	2	12.5

from consideration but again in the absence of basic knowledge concerning humoral antibodies in this disease, we cannot be sure (7)

If the hemagglutination were due to the virus of infectious hepatitis, one might expect that fresh sera from cases of infectious hepatitis would be equally capable of agglutinating red cells. The work on the

masking of the hemagglutinating capacity of the pneumonia virus of mice (8) is a clear example, however, of how a negative material can after certain treatment become positive, and thus we cannot exclude the possibility that this hemagglutination is of viral etiology even though none of 22 fresh sera from 11 individuals with infectious hepatitis agglutinated suitable chicken red cells

Satisfactory controls on sera which have been stored for periods of time at ordinary but slightly variable refrigeration are difficult to obtain. Of thirty sera collected in 1942-1945 from 10 cases of primary atypical pneumonia at the Rockefeller Institute one agglutinated chicken cells in a dilution of 1/200. On the other hand, of 20 sera

TABLE VII  
*Tests for Agglutination in Other Sera*

KIND OF CASE	% PATIENTS	% SERA	% POSITIVE
Infectious hepatitis, fresh sera	11	22	0
Infectious hepatitis, old sera, Rockefeller Inst	10	20	0
Infectious hepatitis, old sera, Johns Hopkins Hospital	3	9	2
Non-infectious jaundice, old sera, Johns Hopkins Hospital	9	16	1
Primary atypical pneumonia, old sera, Rockefeller Inst	10	30	1

from 10 cases of infectious hepatitis, obtained at the same place and time, none agglutinated chicken red cells<sup>1</sup>

Of sixteen sera, about a year old, from nine cases of non-infectious jaundice at the Johns Hopkins Hospital, only one was positive, this a grossly contaminated serum which would have ordinarily been excluded. Of nine one-year old sera from 3 cases of infectious hepatitis, 2 agglutinated chicken red cells in a dilution of 1/80 and 1/160. This data is summarized in Table VII, and unfortunately does not give an unequivocal answer to the question of virus hemagglutination.

#### DISCUSSION

One obvious possibility which has not been dealt with in the text of this paper is that contamination of the sera with bacteria or fungi was

<sup>1</sup> Courtesy Dr F L Horsfall

the cause of the agglutination. This remains a likely explanation but should not be used as the final answer. Only clear sera were used. Of 8 sera which subsequently were shown to contain agglutinins and which were cultured before any work was done, 4 or 50 per cent yielded bacterial growth on sub-culture into Brewer's thioglycollate media and/or on a blood plate. Of 29 sera subsequently shown to be negative for agglutinins, 5 yielded bacteria. In another case an unidentified fungus was found. This latter, in one test, produced red cell agglutinins when grown in normal serum. However, it is well-known that a number of bacteria will agglutinate different red cells and, as indicated above, a number of sera which had a high content of agglutinins were negative on culture. It is true that in these latter sera the bacteria might have died out and the agglutinins have persisted.

Despite this it was felt that the high titer of the substance in the sera, the limitation of the reaction to red cells derived from only certain chickens, and the effect of the type of cleaning of glassware on the reaction justify the report.

#### SUMMARY

A factor capable of agglutinating chicken red blood cells was found in old hepatitis sera. This factor, which would agglutinate only cells from certain chickens, was present in high concentrations, particularly during the first fifteen days of illness. During this time, 8 of 19 (42 per cent) sera were positive in titers of 1/600 to 1/15,000. Thereafter a total of 59 sera yielded six that were positive (10 per cent) with a titer of 1/60 to 1/1260. The pattern agglutination of these chicken red cells was potentiated by traces of soap such as may be left behind after inadequate rinsing but the reaction was regularly obtained in tubes cleaned in chromic acid cleaning solution which was the standard used throughout. The factor was not directly related to the original bilirubin level of the plasma nor to the occasional bacterial or fungous contaminant. A fungus capable of agglutinating red cells after growth in normal serum was obtained in only two of some forty sera that were cultured on Sabouraud's medium but positive cultures were not obtained in 20 cases.

The factor was not sedimented by a gravitational field of 50,000-90,000 for one hour which might mean that it was well below 50  $m\mu$  in size. It did not dialyze through a collodion sac.



It was not found in fresh sera from cases of infectious hepatitis. One of 30 sera from 10 cases of atypical pneumonia was able to agglutinate chicken red cells in a dilution of 1/200. Of sixteen old sera from 9 cases of non-infectious jaundice, only one was positive, this one grossly contaminated with bacteria. On the basis of the above evidence it is possible that this agglutination was caused by the virus of infectious hepatitis which had been unmasked by long storage and/or bacterial growth, but it is more likely that bacterial contaminants or persisting metabolic products of bacteria are responsible.

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# INFLUENCE OF SEX UPON THE LETHAL EFFECTS OF AN HEPATOTOXIC ALKALOID, MONOCROTALINE\*

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In human beings, Laennec's cirrhosis is more than twice as frequent in males as in females (1). This fact has usually been attributed to the greater incidence of alcoholism in males. However, the incidence of cirrhosis is also greater in male adults who profess to be non-alcoholics (2). This suggests that the male sex hormones may in some manner increase the susceptibility of human beings to chronic hepatic damage. No experimental evidence has been published to support this view. On the other hand, the effect of estrogens on liver damage in experimental animals has been studied by Gyorgy and his associates. They reported that female rats were less susceptible than males to the toxic effects of inhalation of carbon tetrachloride (3). Furthermore, estrone partially protected rats against dietetic cirrhosis (4), and particularly when administered in combination with methionine, exerted a striking lipotropic effect (5). These authors reviewed earlier studies which had shown that female rats were less susceptible than males to deficiency of cystine (6) or choline (7), and that female mice were less susceptible than males to the nephrotoxic effects of chloroform (8, 9).

The susceptibility of rats and mice to the effects of chlorinated hydrocarbons and of dietetic deficiency, then, appeared to be influenced by the animal's sex. During a study of the influence of diet upon the lethal effects of monocrotaline, an hepatotoxic alkaloid, it was noted by chance that male rats fed certain of the diets died more quickly than females (10). Because of irrelevant circumstances, these diets, although all qualitatively similar varied somewhat quantitatively in a manner to be described. This fortuitous observation suggested the use of monocrotaline as a tool for the analysis of the relation between sex and the susceptibility of rats to hepatotoxic agents.

\* These studies were conducted under contract with the Office of Naval Research, U S Navy

Monocrotaline was first isolated by Neal and his associates (11) from *crotalaria spectabilis*, a legume used to enrich the soil. *Crotalaria spectabilis* is toxic for cattle (12), swine (13), chickens (14, 15), quail (15), doves (15), horses (16), and goats (16). In general, the administration of *crotalaria* resulted in severe hemorrhagic congestion of the liver with necrosis of those liver cells nearest the central veins. In addition, congestion of the lungs and necrosis of lymphoid tissues were observed (13). This pathologic picture is remarkably similar to that produced by the ingestion of plants of another genus, *senecio* (ragwort or Stinking Willie), a weed responsible for hepatic damage in cattle (17-21), horses (20, 21), rats (22), guinea pigs (22), mice (23), and human beings (22). The similarity between the symptoms of *crotalaria* and *senecio* poisoning apparently has its basis in the chemical nature of their respective alkaloids. Adams and Rogers (24) demonstrated that monocrotaline, derived from *crotalaria*, and alkaloids derived from *senecio* contain, in common, the alkanolamine retro-necine.

Harris, Anderson and Chen (25) observed that monocrotaline was toxic for mice and rats, and that the lesions produced were similar to those which followed the administration of *crotalaria*. The experiments to be described indicate that the susceptibility of rats to monocrotaline is influenced by their age and by hormonal and dietetic factors.

#### METHODS

Hooded rats of the MacCollum hybrid strain were weaned at 21 days and treated as described below. In one experiment, hooded rats of the Harvard strain were used.

*Diet*—The rats were allowed tap water as desired, and fed one of the following diets.

*Diet A* Ralston Purina Dog Chow as desired. This diet consists of meat meal, dried skimmed milk, wheat germ, dried beet pulp, corn grits, cereal feed from corn and wheat, dried figs, soy bean oil meal, molasses, riboflavin supplement, brewer's dried yeast, Vitamin A and D feeding oils, one per cent steamed bone meal, and one per cent iodized salt. According to the manufacturer, an average sample contained 23.0 per cent protein, 6.0 per cent fat, 9.6 per cent ash, and 6.0 per cent crude fiber. In addition, an average sample contained carotene, 9 mg, thiamin 5.6 mg, and riboflavin 5.0 mg per 100 grams.

An excess of food was offered each animal, the amount of food consumed daily was not measured.

Diet B (26) Vitamin-test casein (General Biochemicals, Inc), 4 per cent, dried brewer's yeast, U S P (Fleischman's Type 90B), 16 per cent, powdered corn starch (Argo), 71 per cent, cod liver oil (Peder Devold Company "High Potency"), 1 per cent, peanut oil (Somold, Suffolk Oil Mill, Suffolk, Virginia), 5 per cent, and salt mixture (27), 3 per cent. In addition the diet contained 250  $\mu$ gm of thiamine, 312  $\mu$ gm of riboflavin, 250  $\mu$ gm of pyridoxine, and 1.5 mg of calcium pantothenate in each 100 grams. The ration contained 12 per cent protein and 6.8

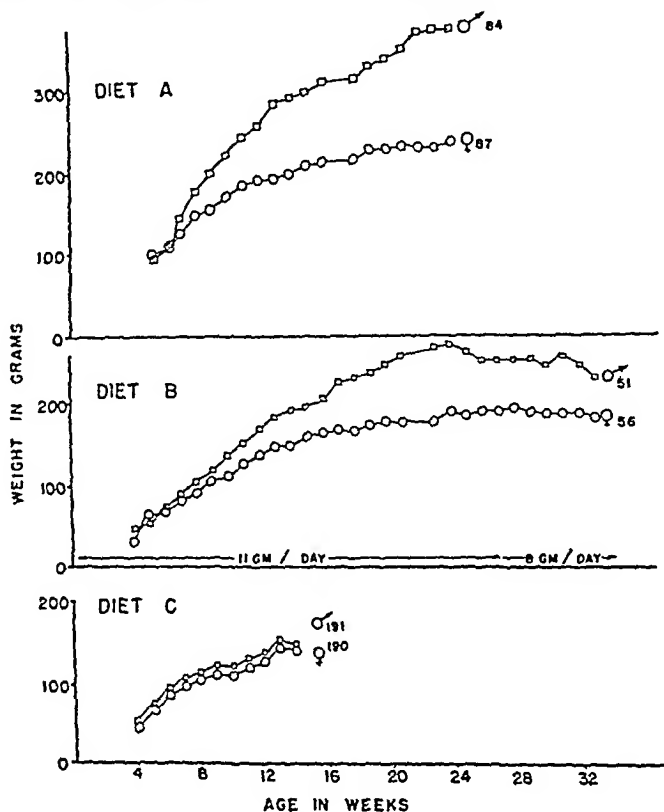


FIG 1 REPRESENTATIVE GROWTH CURVES OF RATS FED FROM WEANING ON DIETS A, B, AND C

per cent fat. The diet was prepared by mixing the oils and casein together, and then adding a mixture of the other dry ingredients. Food for approximately one week was made at one time, and then stored in the refrigerator at 4°C. The animals were fed four times a week. Each was fed a total of 77 grams of diet per week for 8 to 24 weeks after weaning, and then 56 grams per week for 2 to 6 weeks before the first injection of monocrotaline.

Diet C. Diet B, 56 grams per week after weaning for the entire experimental period.

Typical growth curves have been reproduced in Fig 1.

*Housing*—The rats were individually housed. Those on Diet A were kept in cages with wire mesh bottoms. Those on Diets B and C were housed in cages with removable solid bottoms so that food could not be lost through the bottom of the cage.

*Administration of Monocrotaline*—In most experiments, after the preliminary period, rats were injected intraperitoneally every second day with 46 mg of monocrotaline<sup>1</sup> per kilo of body weight. This is half the reported intravenous LD<sub>50</sub> dose (25). The rats were weighed immediately before each injection of monocrotaline and the dose calculated accordingly. During the period of injection, rats on Diet A were allowed food as desired, and those on Diets B and C were fed 56 grams of food per week. The monocrotaline was prepared for injection by dissolving it in an equivalent amount of N/14 hydrochloric acid and diluting the solution to one per cent monocrotaline with an 0.85 per cent aqueous solution of sodium chloride buffered to pH 7.35 with potassium phosphate in M/20 final concentration.

*Technique of Castration*—In experiments in which castrated animals were used, male rats 3 to 5 weeks old were anesthetized with ether and the testes removed transperitoneally through a mid-line lower abdominal incision. Until these male animals were castrated, they and their female litter mates were fed Diet A. Thereafter, all were fed Diet C.

*Statistical Methods*—In each experiment the results were analyzed by the chi-square method, using a four-fold table, and by the standard error of the difference in two means. No differences were considered significant unless the probability that the result was due to chance was less than 1 in 100 (28).

## EXPERIMENTAL

### 1 *Pathologic Effects of Intraperitoneal Injection of Monocrotaline*

When rats were injected intraperitoneally every second day with 46 mg of monocrotaline per kilo of body weight, they differed in their susceptibility to monocrotaline in a manner to be described in succeeding sections. The symptoms and pathologic findings appeared to vary only with the time of death rather than with age, sex, or dietetic history. The principal pathologic changes observed were necrosis of the hepatic parenchyma and renal tubular epithelium. These changes were more extensive in those rats which died within about 10 days. In those which died later, the damage in the liver and kidneys was definite but less severe. These rats had pulmonary congestion and edema, and dilation of the right side of the heart. The presence of ascites, jaun-

<sup>1</sup> Monocrotaline was obtained through the courtesy of Dr. Roger Adams and Dr. K. K. Chen.

dice and biliruria confirmed the histologic observation that hepatic damage was impressive in both the acute and subacute phases. The lymphoid tissues in the spleen, thymus, and lymph nodes were congested with erythrocytes and the lymphocytes had largely disappeared. Relatively little variation in the pathologic picture was noted from animal to animal.

TABLE I  
*Survival Time of Rats Injected with Monocrotaline\**

DIET	STRAIN	SEX	NO OF RATS	AVERAGE WEIGHT**	AVERAGE SURVIVAL TIME
				gm	days
A	MacCollum	♂	21	246	21 7
		♀	18	155	18 8
B	Harvard	♂	7	234	9 0
		♀	4	186	18 7
	MacCollum	♂	11	209	9 3
		♀	7	169	19 3
	Total group B	♂	18	219	9 2
		♀	11	175	19 1
C	MacCollum	♂	9	143	18 3
		♀	15	136	18 6

\* 46 mg per kilo of body weight intraperitoneally every second day

\*\* Average at time of first monocrotaline injection

## 2 *The Influence of Age, Diet, and Sex on Survival Time of Rats Injected with Monocrotaline*

One-half the reported intravenous LD<sub>50</sub> dose (25) of monocrotaline, 46 mg per kilo, was injected every other day intraperitoneally into rats of both sexes fed various diets. The results of these experiments are shown in Table I and Figs 2 and 3.

Female rats fed Diet A (Purina Dog Chow) *ad libitum* were, on the average slightly more susceptible than males to the lethal effects of monocrotaline. However, this difference in susceptibility between the 2 sexes was not statistically significant as tested by the standard error of the difference in the means. Male rats fed this liberal diet were sexually mature and considerably heavier than females. The increased susceptibility of female rats was most apparent in the younger animals (Fig 3). Among these rats, 8 to 12 weeks old at the time of

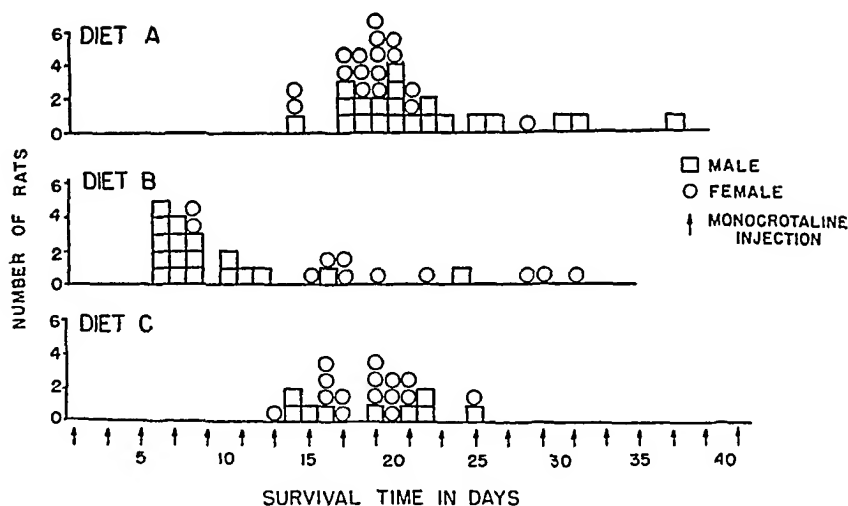


FIG 2 Influence of Diets A, B, and C and of sex upon the survival time of rats injected intraperitoneally every second day with 46 mg of monocrotaline per kilo of body weight. In this and the succeeding figures, each block or circle represents an animal. The survival time after the first injection is indicated in days.

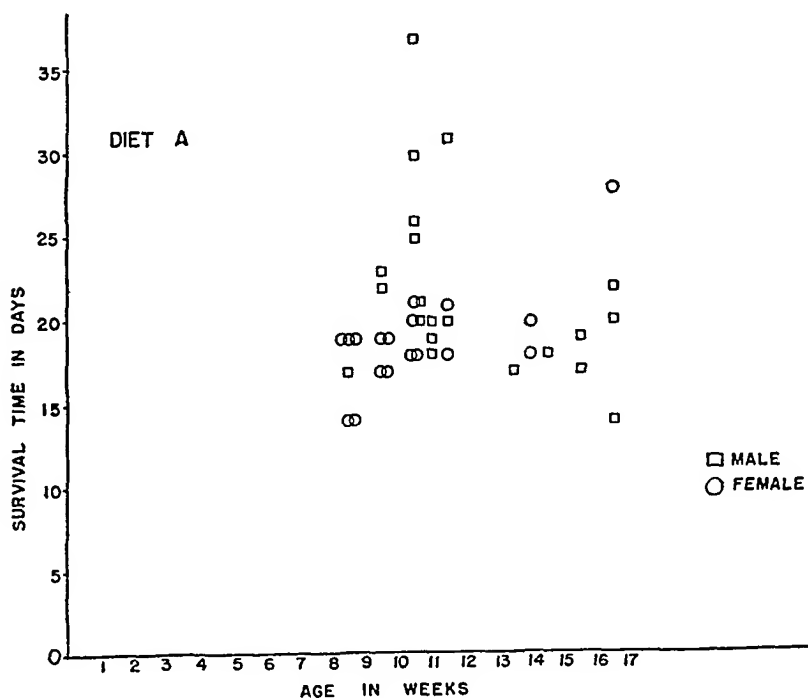


FIG 3 Influence of age upon the survival time of rats on Diet A injected intraperitoneally every second day with 46 mg of monocrotaline per kilo of body weight.

the first injection of monocrotaline, all of 15 female rats died within 21 days, at which time 7 of 14 male rats were still alive. This increased susceptibility of young female rats was statistically significant, as tested by the chi-square method.

When rats were fed from weaning a restricted amount of a diet relatively low in protein (Diet C), both sexes grew slowly and at approximately the same rate. When such rats were injected with half the  $LD_{50}$  dose of monocrotaline, 46 mg per kilo, the survival time was found to be equal in the two sexes, an average of 18.2 days, or approximately the same as that of well-fed normal rats. These rats were 9 to 15 weeks old at the time of the first injection of monocrotaline, the age of the rats did not seem to influence susceptibility.

Some rats, in addition to those already described, were placed at weaning on Diet B, which was moderately deficient. On Diet B the rats grew less than on the complete Diet A. However, in contrast to those on the more deficient Diet C, there was some weight difference between the two sexes. The male rats on this diet weighed an average of 220 grams, and the females 175 grams at the time of the first injection, at which time they were 16 to 34 weeks old. When these rats were injected with half the  $LD_{50}$  dose of monocrotaline, 46 mg per kilo, a striking difference in survival time of the two sexes was noted. The female rats like well-fed males and females, and like deficient males and females survived, on the average, 19.1 days. However, male rats fed this semi-deficient Diet B survived on the average only 9.2 days. The susceptibility of the rats in this group did not seem to vary with their ages within the limits studied.

### *3 The Effect of Weight and Testosterone Propionate on the Survival Time of Rats Injected with Monocrotaline*

At least two possible explanations for the greater susceptibility of male compared with female rats fed Diet B must be considered. Perhaps the greater susceptibility of the males was primarily related to their greater size. Or the greater susceptibility may have been due to some other effect of maleness.

The survival times of rats fed the three different diets and injected with monocrotaline were plotted against their weights at the time of the first injection as shown in Fig. 4. It seems unlikely that differences



in weight *per se* can explain the differences in susceptibility between the sexes in rats fed Diet B. These weight differences were actually much less than in rats fed Diet A where no such sex difference in susceptibility was noted. For this reason, the following experiment was designed to test for some other effect of maleness.

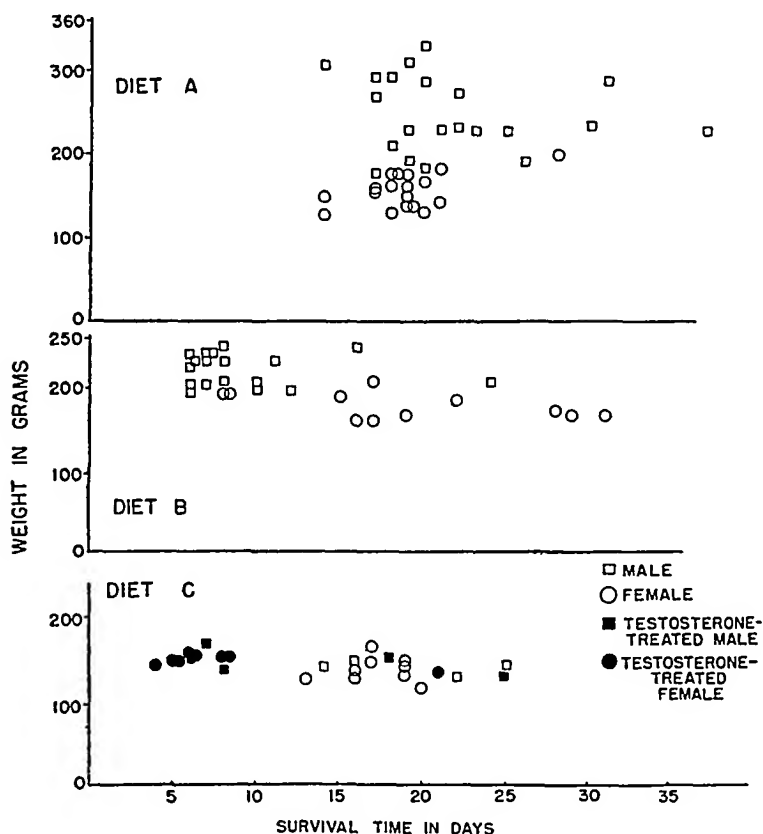


FIG 4 Influence of body weight and Diets A, B, and C upon the survival time of rats injected intraperitoneally every second day with 46 mg of monocrotaline per kilo of body weight

Thirteen male and female rats which had been fed from weaning Diet C for 6 to 8 weeks were injected subcutaneously with 2.5 mg of testosterone propionate in sesame oil (Rare Chemicals, Inc.) daily for 2 weeks. Then each was injected intraperitoneally every other day with 46 mg of monocrotaline per kilo of body weight, while the injections of testosterone propionate were continued. At the same

time, 13 control rats of both sexes from the same litters and on the same diets were injected with monocrotaline alone. Those animals which were to be injected with testosterone propionate weighed, on the average, 128 grams before the first injection of testosterone, and the control rats weighed, on the average, 121 grams at this time. Two

TABLE II  
*Influence of Testosterone Propionate Upon Survival Time of Rats Injected with Monocrotaline\**

GROUP	SEX	NO OF RATS	WEIGHT**	AVERAGE SURVIVAL TIME
			gm	days
Control	♂	4	139	19.2
	♀	9	137	17.3
Testosterone Propionate	♂	5	147	12.8
	♀	8	149	7.9

All rats were fed Diet C

\* 46 mg per kilo of body weight intraperitoneally every second day

\*\* Average at time of first monocrotaline injection

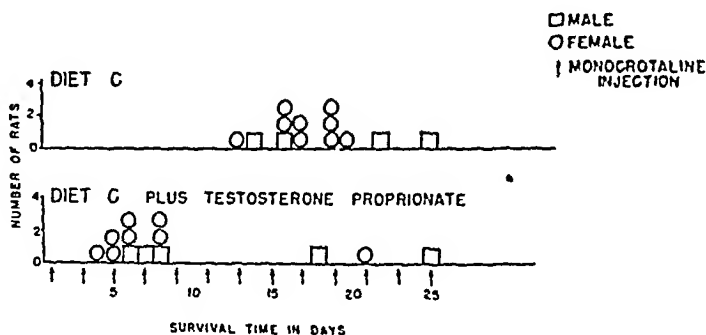


FIG 5 Influence of testosterone propionate upon survival time of rats on Diet C injected intraperitoneally every second day with 46 mg of monocrotaline per kilo of body weight. The rats were injected with 2.5 mg of testosterone propionate daily for 2 weeks before and during the injection of monocrotaline.

weeks later, at the time of the first injection of monocrotaline, the testosterone treated rats weighed on the average, 148 grams, and the controls, 137 grams. In Table II and Figs 4 and 5, it will be seen that injection of testosterone propionate increased the susceptibility to monocrotaline of male and female rats fed a limited diet (Diet C) of purified foodstuffs relatively low in protein. In this experiment,

the testosterone treated rats died, on the average, in 9.8 days, while the control rats survived 18.0 days. Despite the small differences in weight between the testosterone treated and control rats, a dramatic difference was observed in the susceptibility of the animals to the lethal effects of monocrotaline.

#### 4 *The Effect of Castration, of Estradiol Benzoate, and of Methionine on the Survival Time of Rats Injected with Monocrotaline*

It has been shown in the preceding section, then, that under conditions of restricted food intake, *maleness* increased the susceptibility of rats to monocrotaline. The experiments of Gyorgy and his associates

TABLE III

*Influence of Castration and of Estradiol Benzoate Upon Survival Time of Rats Injected with Monocrotaline\**

GROUP	NO. OF RATS	WEIGHT**	AVERAGE SURVIVAL TIME
		gm	days
Untreated ♀	17	116 (91-138)	22.0
Castrated ♂	7	132 (113-150)	20.9
Castrated ♂ Injected with Estradiol benzoate	8	121 (115-124)	20.4

All rats were fed Diet C

\* 46 mg per kilo of body weight intraperitoneally every second day

\*\* Average at time of first monocrotaline injection, range in parentheses

(3-5) indicated that *femaleness* protected rats against injury by diet and by carbon tetrachloride. Therefore, the rôle of estrogens in the resistance of rats to monocrotaline was studied.

Fifteen male rats were fed Purina Dog Chow *ad libitum* (Diet A) after weaning, castrated 2 to 14 days later, and fed Diet C from then on. Starting 19 days after castration, 8 of these rats were injected subcutaneously every other day with 100  $\mu$  gm of estradiol benzoate in sesame oil (Progynon-B, Schering). After 2 more weeks, these 8 rats were injected intraperitoneally every other day with 46 mg of monocrotaline per kilo of body weight, while the injections of estradiol benzoate were continued. At the same time, the other 7 castrated males and 17 litter-mate females which had been fed the same diets

for the same times were injected with monocrotaline in the same manner

The survival times of all these rats are recorded in Table III and Fig 6. It will be seen that castrated male rats survived an average of 20.9 days. This was about the same time as intact males and females fed either the same restricted Diet C, or the complete Diet A.

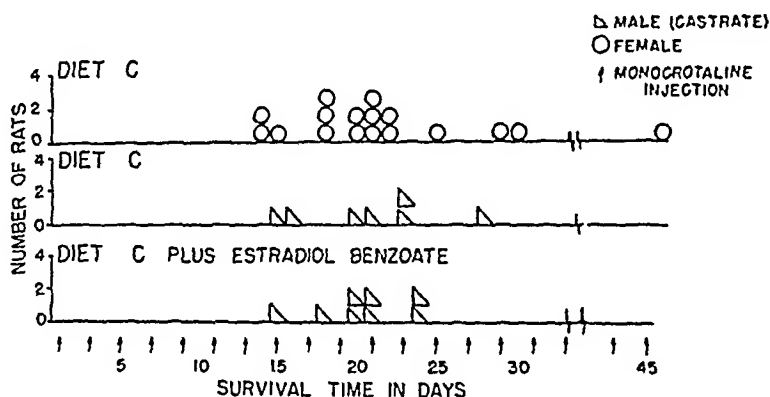


FIG 6 Influence of castration and of estradiol benzoate upon the survival time of rats on Diet C injected intraperitoneally every second day with 46 mg of monocrotaline per kilo of body weight. Estradiol benzoate, 100  $\mu$  gm was injected every second day to castrated male rats for 2 weeks before, and during the administration of monocrotaline.

In this experiment injections of estradiol did not appear to prolong the survival time of castrated male rats injected with monocrotaline.

Methionine has been shown to prevent fatty infiltration of the liver in rats fed diets high in fat (30). Furthermore, methionine has been observed to prevent the hepatic necrosis and hepatic cirrhosis produced in rats by diets low in protein (31, 32). And methionine protected protein-depleted dogs against the toxic effects of chloroform (33) and of mapharsen (34). On the other hand, Drill and Loomis (35) were unable to demonstrate that methionine had any beneficial effect upon the hepatic damage produced by carbon tetrachloride in dogs fed either high or low protein diets. Moreover, it has been shown by Earle, Smull, and Victor (36) that large amounts of methionine apparently caused atrophy of liver cells. It seemed of interest to test the effect of methionine on the toxicity of monocrotaline.

The effect of daily subcutaneous injections of 60 mg of dl-methionine (Merck) in 3 per cent solution was tested in two groups of rats. The

methionine was dissolved in M/20 potassium phosphate aqueous buffer (pH 7.35) containing 0.85 per cent sodium chloride. In the first experiment, the administration of methionine was started simultaneously with the intraperitoneal administration every other day of 46 mg of monocrotaline per kilo of body weight to rats which had been fed the more restricted Diet C for 9 weeks. In the second experiment, the methionine was injected daily for 7 days before, and during the course of monocrotaline, and simultaneously the control rats were injected with the same volume of buffer. These rats had been fed Purina

TABLE IV  
*Influence of Methionine Upon Survival Time of Rats Injected with Monocrotaline\**

REGIMEN	NO OF RATS	SEX	WEIGHT**	AVERAGE SURVIVAL TIME
			gm	days
A Control (monocrotaline alone)	2	♂	171 (164-179)	18.5
	3	♀	143 (133-158)	21.7
	2	♂	164 (157-172)	10.0
	3	♀	144 (133-161)	16.3
B Control (buffer and monocrotaline)	5	♂	130 (121-142)	13.8
	5	♀	129 (115-140)	20.0
	6	♂	135 (125-148)	10.8
	5	♀	129 (98-143)	14.8

All rats were fed Diet C from weaning except 8 rats, in Group B, which were fed Diet A for 12 days, and then Diet C.

\* 46 mg per kilo of body weight intraperitoneally every second day.

\*\* Average at time of first monocrotaline injection, range in parentheses.

Dog Chow (Diet A) for 3 to 12 days after weaning, and then the more restricted Diet C for 6 weeks before the first injection of monocrotaline.

Under the conditions of these experiments, it was not possible to demonstrate that methionine influenced the length of survival of rats injected with monocrotaline. It is of great interest that, compared with simultaneous controls, there was considerably less necrosis in the livers of rats injected with methionine, despite its apparent failure to prolong life. It is possible that the administration of methionine in different dosages or by different routes might have influenced the susceptibility of rats to monocrotaline.

In the second experiment with methionine, male rats were more

susceptible than females in both the experimental and control groups. This increased susceptibility occurred only in the 8 rats which had been fed Diet A for 12 days after weaning and Diet C from then on. These rats were equally divided between the experimental and control groups and their inclusion in the experiment did not appear to influence the outcome. No significant difference was noted between the weights of male and female rats in this experiment.

### 5 *The Effect of Larger, Daily Injections of Monocrotaline in Rats Fed Purina Dog Chow*

Five male and 5 female rats, 17 to 31 weeks old, which had been fed Purina Dog Chow (Diet A) from weaning, were injected intraperi-

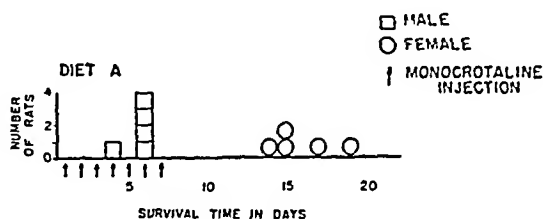


FIG 7 Delayed lethal effect in rats on Diet A of seven daily intraperitoneal injections of 92 mg of monocrotaline per kilo of body weight

toneally daily for 7 days with 92 mg of monocrotaline per kilo of body weight. This amount is the reported intravenous  $LD_{50}$  dose (25). The male rats averaged 341 grams in weight, and the females 199 grams at the time of the first injection. The amount of food consumed was not measured, but it was apparent that the animals ate much less after the first injection than earlier.

One male rat died on the fourth day, and the other 4 on the sixth. At this time, all the females were still alive (Fig 7). The daily injections of monocrotaline were discontinued after 7 days. The female rats lost weight gradually, and died from 14 to 19 days after the first injection, an average of 16 days later. The gross and microscopic picture in these rats could not be distinguished from other rats which died at this time after injections of 46 mg of monocrotaline per kilogram of body weight every second day. Thus it appeared that the same sex differences previously noted in moderately deficient rats injected with monocrotaline were apparent in initially well-nourished

animals injected with larger, more frequent doses of this alkaloid. Moreover, a delayed lethal effect was noted in rats in which the administration of the alkaloid had been discontinued.

### 6 *The Delayed Effect of Relatively Small Doses of Monocrotaline*

Twelve male and 6 female rats were fed the more restricted moderately low protein diet (Diet C) for 12 to 14 weeks and then given 5 injections of 23 mg of monocrotaline per kilo of body weight at twice-weekly intervals. During the first few days succeeding the last injection of monocrotaline several of the rats failed to finish their daily diet of 8 grams. During this period, the amount of food offered each of the other rats was curtailed to equal the amount eaten by these animals. Sixteen days after the first injection, 2 male rats died. All

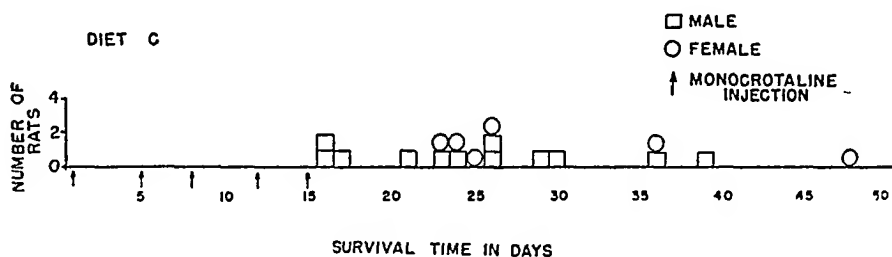


FIG 8 Delayed lethal effect in rats on Diet C of 5 twice-weekly intraperitoneal injections of 23 mg of monocrotaline per kilo of body weight

the remaining animals died at intervals thereafter, the last 48 days after the first injection. On the average, the male rats survived 25.2 and the females 20.3 days after the first injection of monocrotaline (Fig 8). This difference was not statistically significant.

The pathologic picture observed in these rats which died some time after the last injection of monocrotaline did not differ from that of rats which died at the same time after injection every second day with 46 mg of the alkaloid per kilo of body weight. As in the previous experiment, death occurred at a considerable interval after the administration of monocrotaline had been discontinued. It is of note that with this much smaller dosage of alkaloid the average survival time of these rats was only moderately prolonged compared with rats given 46 mg of monocrotaline per kilo of body weight every second day.

## DISCUSSION

The experiments described demonstrated that the susceptibility of rats to the lethal effects of monocrotaline was influenced by their age and sex, the particular diet they ate, and the dosage of the alkaloid injected. Variations in any of these factors changed the relative susceptibility of the animals. Under certain conditions of age, diet, and dosage, male rats were more susceptible than females to the lethal effects of monocrotaline. This increased susceptibility could be induced in female rats by the administration of testosterone propionate. Castrated male rats, however, were as resistant as females, and the administration of estradiol benzoate to these castrated rats did not afford increased protection.

The mechanism by which *maleness* or the administration of testosterone propionate increased the susceptibility of rats to monocrotaline is not clear. We have made no studies to observe whether an anatomic basis exists for the difference in the susceptibility of the two sexes. This has been suggested to explain the greater susceptibility of male mice to chloroform (9, 37). However, the male rats' nutritional status was of the greatest importance in determining the duration of survival. The sexual difference in susceptibility was evident only in those animals which were first fed a diet such that male rats grew larger than female, and then were fed, before the administration of monocrotaline, a more deficient diet (Sections 2 and 4). Or, in one experiment with well nourished rats, sufficient monocrotaline was administered to produce anorexia and consequently an acute deficiency state (Section 5).

Either acute or chronic dietetic restriction, then, in sexually differentiated rats, increased the susceptibility of the male to the lethal effects of an hepatotoxic alkaloid. Since fully grown male rats are larger than females, one would expect that when both sexes are fed equal, restricted amounts, the males would have relatively less food available than the females. The greater size of the males, however, was not the only determining factor in susceptibility. Both male and female rats injected with testosterone propionate were more susceptible than control rats of the same weight which were fed the same quantities of the same diet. This was true even though relatively large



changes in the dosage of monocrotaline effected relatively small changes in the survival time (Section 6)

The increased susceptibility of deficient male or testosterone treated rats, therefore, did not seem related necessarily to the greater size of the males. Perhaps under the conditions of these experiments, the composition of the various tissues was modified by the androgens. Biochemical differences between male and female rats have been noted (38-43), and the androgens have been reported to have metabolic activities not necessarily related to their primary sexual effects (44-47). Conceivably, under the influence of the androgens, nutrients needed for protection against monocrotaline were diverted to other uses. This diversion of nutrients may explain the greater susceptibility of male rats, or rats injected with testosterone propionate, compared with females, to the lethal effects of monocrotaline. However, if a surplus of food were provided, sufficient amounts of the essential nutrients would be available for both purposes.

When the conditions of the experiment were changed, the relative susceptibility of male and female rats was also changed. For example, age seemed an important factor. Among young rats, 8 to 12 weeks old, fed Purina Dog Chow (Diet A), females were more susceptible than males to the effects of injections of monocrotaline, a difference not observed in older rats on this diet. This was the reverse of what was found in rats fed a relatively low protein diet (Diet B) or injected with testosterone propionate.

In certain experiments, the rats died 1 to 23 days after the last injection of monocrotaline. This same phenomenon of death after a latent period has been described repeatedly in the literature in livestock which have eaten *crotalaria*. This suggests either that significant amounts of monocrotaline, or an active fraction, are retained in the body, or that it initiates irreversible changes in the animal's metabolism.

The experiments reported emphasize the important influence of age, sex, and diet upon the susceptibility of rats to hepatotoxins. Undoubtedly, other factors, not specifically studied, also influence susceptibility. The data reported here emphasize the importance of relatively small changes in the design of an experiment in determining its outcome.

## SUMMARY

(1) The influence of age, sex, and diet upon the lethal effects of monocrotaline, an alkaloid derived from *crotalaria*, has been reported. Under the conditions studied, the administration of monocrotaline to rats resulted in necrosis of the liver and renal tubules, congestion of the lymphoid tissue with erythrocytes, and, in rats which died after about ten days, pulmonary congestion and edema.

(2) In general, no difference was noted in the survival time of male and female rats fed a supposedly normal diet and injected with monocrotaline. However, if only rats 8 to 12 weeks old were considered, females survived a shorter time than males.

(3) Male rats, fed a relatively low protein diet, died in approximately half the time of females fed the same diet or males and females fed the normal diet.

(4) Rats of both sexes fed a still more deficient diet were as resistant as normal rats.

(5) The administration of testosterone propionate increased the toxicity of monocrotaline for both sexes on the more deficient diet. This was true even though testosterone-treated and control rats weighed approximately the same amount. Neither castration nor the administration of estradiol benzoate influenced the survival time of rats on this latter diet.

(6) The experiments suggest that the greater susceptibility of deficient male rats to monocrotaline may be due to the metabolic effects of male sex hormones.

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# ATTEMPTED HETEROLOGOUS TRANSPLANTATION OF CARCINOMA OF THE CERVIX

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Greene (1, 2, 3) reported several instances of successful heterologous transplantation of malignant tumors into the anterior chamber of the guinea pig's eye using human carcinoma and sarcoma tissue. Hovenarian and Deming (4) obtained growth in the guinea pig's eye using human prostatic carcinoma which had metastasized to the inguinal area. Greene felt that this biologic test demonstrated autonomy of the malignant tissue in a more exact manner than obtainable by clinical and microscopic studies. Autonomy of malignant tissue is present when this tissue can invade and live in a foreign tissue, a tissue other than the parent of the neoplasm. Using this biologic concept of malignancy and autonomous growth, Greene would question the usual microscopic diagnosis of malignancy based upon anaplastic cellular changes and evidence of local tissue invasion or lymph vessel and lymph node spread.

To discount the microscope, to alter established microscopic evidence, and to accept heterologous transplantation as the only method to determine autonomous growth goes against the teachings and beliefs of most pathologists. It makes the diagnosis entirely too rigid and would delay rather than enhance the all important efforts at early diagnosis and treatment of cancer. As a method for studying malignant tissue, it has appealing advantages.

Carcinoma of the cervix presents two particular problems which successful intraocular heterologous transplantation might assist in solving. It has been recommended that epidermoid carcinoma of the cervix be divided into spinal, transitional, and basal types according to the preponderant microscopic cell type. The unconfirmed, but general impression exists that spinal cell carcinoma of the cervix is less radiosensitive and less rapid in its spread and basal cell carcinoma more radiosensitive and more rapid in its clinical advance. Since repeated

cervical biopsies or even a single biopsy in separate areas will show an entirely different type of cell, most of the cases have been lumped into the middle category as transitional. Greene has shown that the tissue of origin becomes more evident by heterologous transplant study, and it was hoped, that if such a method were successful using human cervical carcinoma, the preponderant epidermoid cell type could be determined. The second problem deals with the earlier stages of cervical malignancy, the intraepithelial, pre-invasive, or carcinoma-in-situ stage about which there has been so much discussion. Galvin and TeLinde (5) felt that this represented an early stage of true carcinoma and their large series of cases suggested this conclusion from a microscopic angle. Although Greene termed this type of lesion precancerous, he found similar lesions of unstated origin incapable of growth in anterior chamber transplant experiments. A successful biologic test would do much to clarify this microscopic interpretation.

#### MATERIAL AND METHOD

Twelve patients with carcinoma of the cervix which had advanced beyond the confines of the cervix were used. Three cases were classified as League of Nations II, seven as League of Nations III, and two as League of Nations IV. None had begun treatment and cases which by examination and biopsy showed extensive necrosis or overwhelming infection were not used. All of the above biopsies showed a heavy epithelial component and a not unusual amount of desmoplastic reaction.

Biopsies of the carcinoma were taken in as papillary an area as possible and these were placed in a sterile petri dish containing distilled water with 500 to 1,000 units of penicillin per cc. Transplants were done within three hours after the biopsy was secured.

Eight adult female guinea pigs were used for each biopsy. Using sterile precautions the eye was locally anesthetized with pontocain solution and the anterior chamber was entered by means of a corneal knife incision at the corneoscleral junction. Through this incision a trocar containing tissue with about a 0.5 mm diameter was inserted and the tissue guided to the lower iris angle by a blunt instrument pressed externally.

## RESULTS

A foreign body reaction associated with some infection was a prominent feature during the first six to ten days of observation in all cases. The eyes of all animals in one case and two to six animals in most of the cases never recovered from the infection, the transplant hyalinized eventually and the eye became completely clouded. In all but one

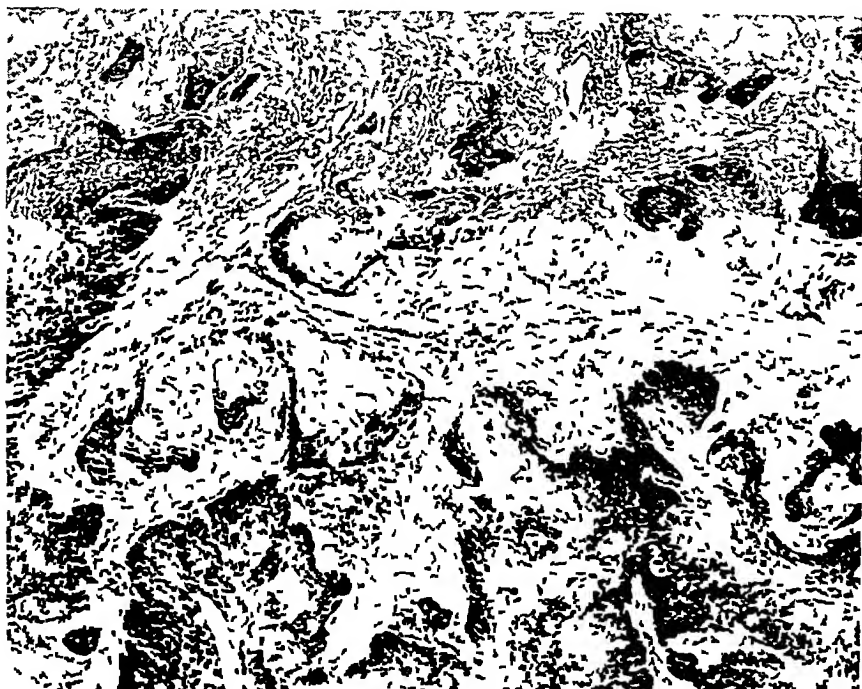


FIG. 1 Epidermoid carcinoma of the cervix, spinal cell type, in a biopsy taken at the same time that tissue was obtained for anterior chamber transplantation. Note the pearl formation.

case two to four of the eyes in each group of eight guinea pigs showed an apparently healthy transplant with beginning vascularization of the tissue by twelve to fourteen days. At no time was there definitely discernible growth and by nineteen to twenty-eight days the apparent "takes" had become smaller and almost a dead white in color. The animals have been observed from six weeks to fourteen months without evidence of delayed or slow growth.

In several instances the eye was sacrificed for microscopic study

after it was evident that there was a diminution in size and devascularization. Only two eyes in two separate cases were found to contain viable malignant tissue, twenty-two and fifteen days respectively after the original transplantation. Both fragments were quite small. One case was classified as a spinal cell type of epidermoid carcinoma by biopsy (Fig 1) and the transplant did show the formation of a pearl



FIG 2 Carcinoma with pearl foundation still viable in the anterior chamber of guinea pig's eye twenty-two days after transplantation of the type of tissue shown in Fig 1

twenty-two days later (Fig 2). The other case was transitional by biopsy (Fig 3) and fifteen days later the transplant was a similar cell type (Fig 4). Microscopic studies of other guinea pigs' eyes six weeks to several months after attempted transplant revealed no malignant tissue. A few serial transplants were attempted from fourteen to twenty-one days after the original transplant, but none of these even gave a suggestive "take". The lack of primary growth made such serial transplanting impractical.



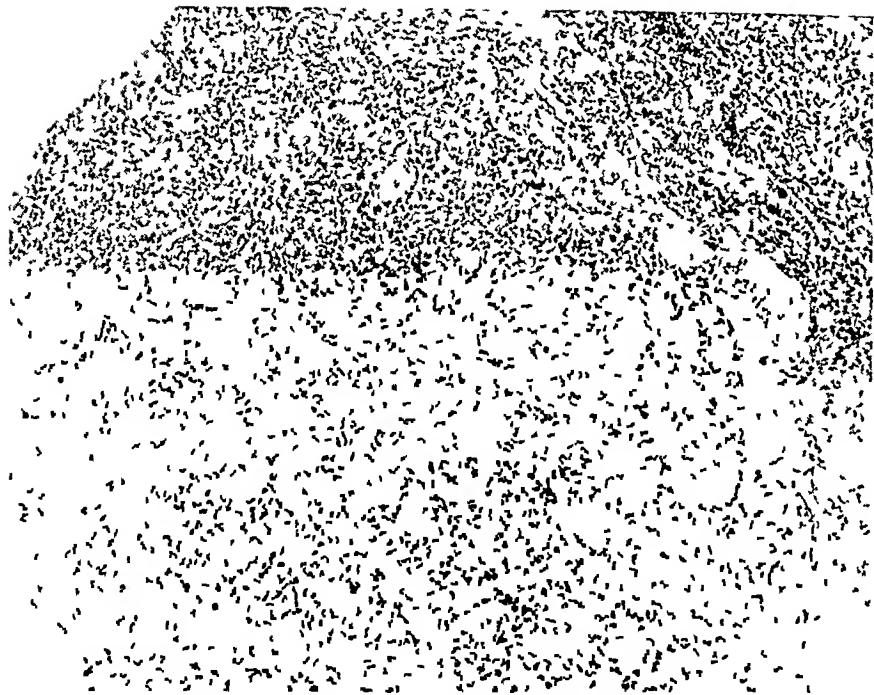


FIG 3 Epidermoid carcinoma of the cervix, transitional cell type, in a biopsy taken at the time of securing tissue for heterologous transplantation



FIG 4 Microscopic section of a guinea pig's eye fifteen days after transplantation of human cervical carcinoma of the same type and from the same case as shown in Fig 3

## SUMMARY AND CONCLUSION

Heterologous transplantation of human carcinoma of the cervix into the anterior chamber of a guinea pig's eye was attempted. It was hoped that this would demonstrate a preponderant epidermoid cell type (spinal, transitional, or basal) more accurately than the microscope. If these transplants were successful, the experiment could be extended to the intraepithelial carcinoma or carcinoma-in-situ group of cervical epithelial changes.

Twelve untrated and relatively late or far-advanced cervical carcinomas were selected. Care was used to obtain tissue as rich in epithelial cells as possible and with a minimum of infection, necrosis, and fibrotic response. None of the transplants were successful, but in two instances tiny islands of viable malignant tissue were found in the guinea pig's eye fifteen and twenty-two days respectively after the original transplant. One of these cases, a spinal cell type by original biopsy, generated a pearl in the transplant.

It is concluded that the heterologous transplantation technique for the study of human malignant tissue is not applicable to human carcinoma of the cervix. The unavoidable associated secondary infection of the cervical neoplastic tissue and the variable, but ever-present fibrous scarring are probably responsible for the failure of the method.

*Note* This study was made possible by a grant from Mr and Mrs George W. Frederick, Baltimore, Maryland.

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# OBSERVATIONS ON THE EFFECTS OF TETRAETHYL PYROPHOSPHATE (TEPP) IN MAN, AND ON ITS USE IN THE TREATMENT OF MYASTHENIA GRAVIS<sup>1</sup>

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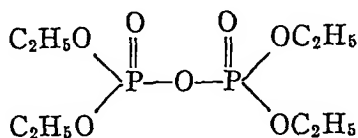
Several esters of phosphoric acid are potent inhibitors of the cholinesterase (ChE) enzymes and as a result have important pharmacological properties. One of these esters, di-isopropyl fluorophosphate (DFP), has been shown by Adrian and his co-workers (1) to inhibit ChE irreversibly. The administration of this compound to human subjects resulted in characteristic effects attributable to the depression of ChE activity in the tissues (2-5). In addition to the usual muscarinic and nicotinic effects of cholinergic drugs, central nervous system symptoms were prominent following the administration of DFP, and were accompanied by easily recognizable electroencephalographic changes. The effect of DFP on neuromuscular function, however, was not as striking following intravenous or intramuscular administration to either normal or myasthenic subjects as one might expect from its potent action in inhibiting ChE. In contrast, when DFP was administered by direct arterial puncture, the nicotinic effects in the muscles supplied by the injected artery were very striking and prolonged. This difference, and the development of central nervous system symptoms following the administration of DFP, were in contrast to the effects of neostigmine administration. This suggested that the solubility properties of these drugs might be important in determining the pattern of their physiological effects, as had been proposed for various neostigmine analogues by Schweitzer, Stedman and Wright (6). As a result, a search was made for other phosphoric acid derivatives which were more highly soluble in water and at the same time retained the ability to inhibit ChE strongly. It was postulated that such substances would have a much more marked effect on neuromuscular conduction than DFP, with less disturbance of central nervous system

<sup>1</sup> The work described in this paper was carried out under a contract between the Medical Division, Chemical Corps, U S Army, and the Johns Hopkins University

function, and might be valuable agents in the treatment of myasthenia gravis (5)

Tetraethyl pyrophosphate (TEPP) was thought worthy of investigation from this point of view and has been under study in this laboratory for the past year. A brief communication has already been made outlining the results (7). At the same time studies with this drug have been carried out in England and have been the subject of preliminary reports (8, 9).

TEPP was prepared as early as 1854 (10) and its chemical structure is believed to be the following (11)



During the war the Germans introduced this substance as an insecticide and it is now used for this purpose (12). TEPP, and the related hexaethyl tetraphosphate (HETP), were found to be potent anticholinesterase agents, with more activity than DFP against rat ChE both in vivo and in vitro (13, 14). When administered to mammals they produced muscarinic, nicotinic and central nervous system effects which resembled those seen following DFP (8, 15).

This communication will describe the results of the administration of TEPP to 18 normal human subjects and to 12 patients with myasthenia gravis. Observations will be reported on the effect of TEPP on ChE enzymes in vitro and in vivo, the general systemic effects of TEPP, and its effect on the gastro-intestinal tract, the central nervous system, and the peripheral neuromuscular unit.<sup>2</sup> Also, the results of its use in the treatment of 12 patients with myasthenia gravis will be discussed.

*The Effect of TEPP on the Cholinesterase Activity of Plasma, Red Blood Cells, Muscle and Brain in Vitro*

Human plasma ChE was more sensitive to the inhibitory effect of TEPP than the cholinesterases present in muscle, brain, and red blood cells, which were less, and approximately equally sensitive (Fig. 1).

<sup>2</sup> The method of making ChE determinations and the techniques for the various physiological studies have been outlined in detail in the studies previously reported on DFP (2-5)

The relation between the concentration of TEPP and the degree of inhibition of ChE activity was linear except for concentrations that caused over 90 per cent inhibition. TEPP was a more potent inhibi-

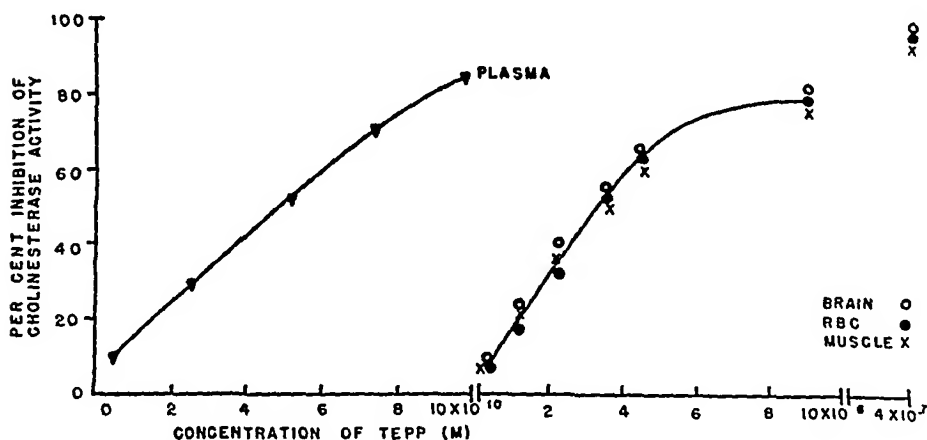


FIG 1 The inhibition of plasma, brain, red blood cell, and muscle cholinesterase activity by TEPP in vitro. The concentrations of the enzyme preparations are as recorded in Table I

TABLE 1

*The in vitro Sensitivity of Human Plasma, Red Blood Cell, Brain, and Muscle Cholinesterases to Inhibition by TEPP, DFP, and Neostigmine*

The molar concentration of each anticholinesterase agent is recorded which produced 50 per cent inhibition of the ChE activity of a solution or homogenate which hydrolyzed  $4.78 \times 10^{-4}$  mM acetylcholine bromide per hour in the absence of inhibitor. This rate of hydrolysis was produced by 51 mg/ml of plasma, 124 mg/ml of brain (cerebral cortex) homogenate, 58 mg/ml of muscle (psoas) homogenate, and 34 mg/ml of red blood cell contents. The initial concentration of acetylcholine bromide was 0.015 M. ChE and anti-ChE compounds were mixed 45 minutes before the addition of acetylcholine.

	PLASMA ChE	BRAIN ChE	MUSCLE ChE	RED BLOOD CELL ChE
TEPP	$5 \times 10^{-9}$	$3.2 \times 10^{-8}$	$3.5 \times 10^{-8}$	$3.5 \times 10^{-8}$
DFP	$9.5 \times 10^{-9}$	$3 \times 10^{-7}$	$2.5 \times 10^{-7}$	$4 \times 10^{-7}$
Neostigmine	$4 \times 10^{-8}$	$4 \times 10^{-7}$	$3 \times 10^{-7}$	$8 \times 10^{-7}$

tor of all four enzymes than was DFP or neostigmine (Table 1). All the anticholinesterase agents were more effective against plasma ChE than against the other human ChE enzymes.

The inhibition of any of the above ChE enzymes by TEPP was not

an instantaneous reaction, but occurred over a period of minutes at a rate which was more rapid at higher concentrations of TEPP (Table 2), and at lower concentrations of enzyme. The combination of TEPP and ChE could not be reversed *in vitro* by dilution at 23°C within the first four hours (Table 2), in contrast to the transient rever-

TABLE 2

*Inactivation of Cholinesterase (Red Blood Cell) by TEPP at Varying Periods of Incubation at 23°C, and Relationship to Concentration of TEPP*

Acetylcholine and sodium bicarbonate were added for the determination of ChE activity after incubation of TEPP with the red blood cell solution. The final concentrations of acetylcholine and of ChE in the dilution experiments were the same as in Table 1, and in the undiluted series fourteen times as great.

The inactivation of ChE was slower when the concentrations of ChE and TEPP during incubation were lower. Fourteen-fold dilution of ChE and TEPP after incubation did not result in restoration of enzyme activity during the period of observation. Similar results were obtained with plasma, muscle and brain cholinesterases.

TEPP AND ChE	UNDILUTED	DILUTED 14 TIMES	
		After incubation	From the start
TEPP conc (M) during incubation	$3 \times 10^{-7}$	$3 \times 10^{-7}$	$2.17 \times 10^{-8}$
TEPP conc (M) when ChE was determined	$2.95 \times 10^{-7}$	$2.1 \times 10^{-8}$	$2.1 \times 10^{-8}$

TIME OF INCUBATION OF TEPP AND ChE	INHIBITION OF ChE ACTIVITY		
minutes	per cent	per cent	per cent
0*	90	8	10
5	95	77	32
10	96	90	51
15	97	90	60
30	95	94	71
45	95	94	90
240	96	96	96

\* TEPP added to ChE at the same time as acetylcholine

sibility of ChE inhibition by DFP, which has been demonstrated over a narrow range of inhibitor concentration during the first hour (16). However, whereas the subsequent inhibition of ChE by DFP is permanent (16, and Table 3), some restoration of ChE activity following inhibition by TEPP was observed after standing for 20 or more hours (Table 3). This restoration of ChE activity was very slight at 5°C,

more marked at 37° than at 23°C, though always incomplete, and somewhat more marked after dialysis. Since the half hydrolysis time of TEPP (in distilled water) is eight hours at 23°C, and four hours at 37° C (17), the time relations suggest the possibility that the slow hydrolysis of any free TEPP might gradually shift the equilibrium in the direction of dissociation of the TEPP-ChE complex. The presence of an enzyme in the tissues capable of accelerating the hydrolysis of TEPP

TABLE 3

*The Effect of Prolonged Standing and of Dialysis on the Inhibition of Cholinesterase (Red Blood Cell) by TEPP, DFP, and Neostigmine*

Acetylcholine and sodium bicarbonate were added for the determination of ChE activity after the indicated periods of time. The concentrations of acetylcholine and ChE were the same as in Table 1. The presence of toluene (0.25 cc) completely inhibited bacterial growth.

Following inhibition by TEPP the ChE activity could be partly restored by prolonged standing, especially at 37°C or with dialysis. In contrast, inhibition by DFP was permanent, while inhibition by neostigmine was completely reversed by dialysis. The presence of neostigmine protected the ChE against inhibition by TEPP. Similar results were obtained with brain, muscle, and plasma cholinesterases, except that restoration of activity of the latter following inhibition by TEPP was less.

	CONCENTRATION (M)	PER CENT INHIBITION OF ChE ACTIVITY AFTER STANDING FOR				
		1 hr at 23°C	96 hrs at 5°C	24 hrs at 23°C	24 hrs at 37°C	24 hrs at 23°C (with dialysis)
TEPP	$9 \times 10^{-7}$	94	94	78	64	60
DFP	$7 \times 10^{-6}$	92	95	97	88	90
Neostigmine	$6 \times 10^{-6}$	90	75	90	70	0
Neostigmine and TEPP*	$6 \times 10^{-6}$	96	78	95	90	8
	$9 \times 10^{-7}$					

\* TEPP added 30 minutes after neostigmine

or the dissociation of the TEPP-ChE complex has not yet been demonstrated, but is suggested by the observation that restoration of ChE activity following depression by TEPP occurs to a somewhat greater extent in red blood cell, brain, and muscle homogenates than in plasma, and considerably more rapidly and more completely in vivo than in vitro.

As had been demonstrated for DFP (18), TEPP was incapable of inactivating ChE which was already in combination with neostigmine. Such a protective action was demonstrated by adding TEPP to ChE

which had been almost completely inactivated by neostigmine. When uncombined TEPP was then removed by hydrolysis and dialysis, and the neostigmine by dialysis, the ChE resumed its original activity. This was in contrast to ChE which had been inactivated by TEPP without prior protection by neostigmine (Table 3).

*The Water-Lipoid Partition of TEPP, DFP and Neostigmine*

The physiological effects of anticholinesterase agents are determined not only by their potency as inhibitors of specific cholinesterases and by the persistence of this inhibition, but also by their ability to reach these various cholinesterases. One important factor determining this penetration may be their relative solubility in aqueous and lipid solutions.

TABLE 4

*The Water-lipoid Partition of TEPP, DFP, and Neostigmine*

The per cent of each anticholinesterase agent extracted from aqueous solution by an equal volume of peanut oil is recorded. The concentrations of anticholinesterase agents and of ChE are the same as in Table 1.

	PLASMA ChE	MUSCLE ChE	RED BLOOD CELL ChE	BRAIN ChE
TEPP	0	14	0	9
DFP	84	87	90	85
Neostigmine	11	15	16	20

vents. Compounds which are more soluble in water might be expected to reach muscle ChE in higher concentrations than brain ChE, which may exist in a lipid phase. Conversely, compounds which are more soluble in lipid might be expected to reach brain ChE in higher concentration. The water-lipoid partitions of TEPP, DFP, and neostigmine were compared by thoroughly mixing aqueous solutions of these compounds with peanut oil, removing the peanut oil by centrifugation, and determining the anti-ChE activity of the remaining aqueous solution. The concentration of TEPP in the aqueous phase was only slightly reduced by extraction with peanut oil, that of neostigmine a little more, while that of DFP was very markedly reduced (Table 4). The DFP removed from the aqueous phase could be demonstrated quantitatively in the lipid phase by the effect of the peanut oil *in vivo* on the ChE activity of plasma and red blood cells.



*The Effect of TEPP on Plasma and Red Blood Cell Cholinesterase Activity in Vivo*

Studies were made following the administration of this drug to human subjects orally, intramuscularly, intravenously and intra-arterially. For intravascular administration the TEPP was in aqueous solution which was freshly prepared for each experiment, as it hydrolyzes in water within several hours (17). Intramuscular injections were made in aqueous solution, in peanut oil, and in propylene glycol. TEPP is stable for many weeks in the latter two vehicles, which were also used

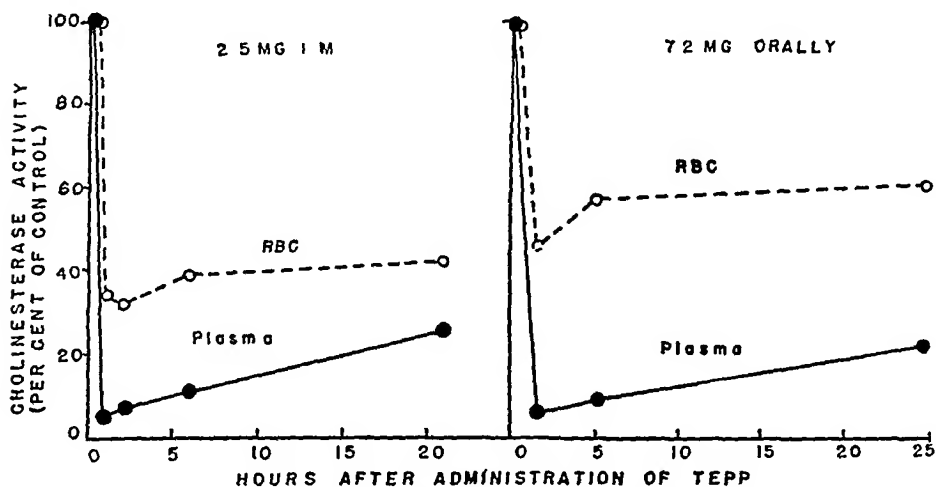


FIG 2 The effect on plasma and red blood cell cholinesterase activity of a single dose of TEPP administered intramuscularly (in aqueous solution) and orally (in propylene glycol) (Subject G R)

for oral administration. The absorption of the drug from the gastrointestinal tract was better when dissolved in propylene glycol. For oral administration a concentration of 5 mg TEPP per cc of propylene glycol was employed. This solution was measured into water by means of a calibrated dropper immediately prior to administration.

The intramuscular or intravascular administration of 1 mg or more of TEPP resulted in a rapid depression of the ChE activity of the plasma to near zero, and of the red cell ChE activity to 60 per cent or less of that originally present (Figs 2 and 3). Approximately four times as large a dose was required to produce a similar effect when the compound was administered orally. As would be expected from the stud-

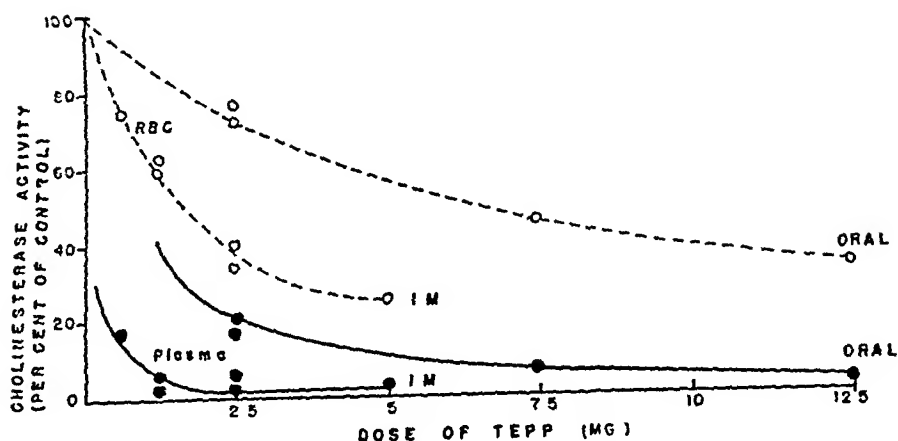


FIG 3 The effect on plasma and red blood cell cholinesterase activity of graded single doses of TEPP administered intramuscularly (in aqueous solution) and orally (in propylene glycol)

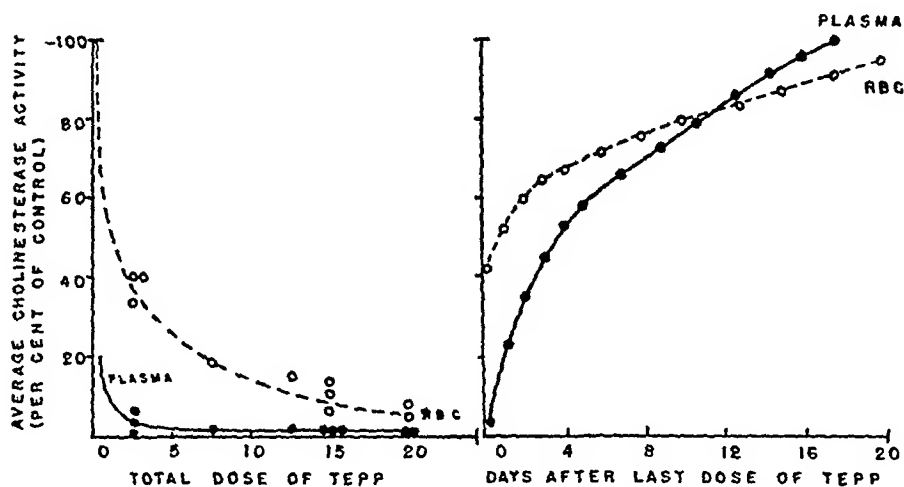


FIG 4 The effect of the daily intramuscular administration of TEPP (2.5 mg qd in aqueous solution) on plasma and red blood cell cholinesterase activity, and recovery after cessation of TEPP (Average values obtained in 4 subjects (left) and 14 subjects (right))

ies in vitro (Fig 1) TEPP had a more striking affinity for plasma ChE, causing a depression to 40 per cent of its original activity before there was any significant effect on red cell ChE

Single graded, or successive doses of TEPP administered by any route produced increasing depression of the ChE activity of plasma

and red cells toward zero (Figs 3 and 4). Each increment of TEPP had a progressively decreasing effect which was proportional to the level of ChE activity existing at the time of that particular dose. Although the depression of both plasma and red blood cell ChE was more marked after the administration of TEPP than of DFP, the relationship between the dosage of TEPP and its effect on ChE activity was quite similar to that encountered with DFP (2). As pointed out by Riker and Wescoe for DFP (19), this relationship is comparable with

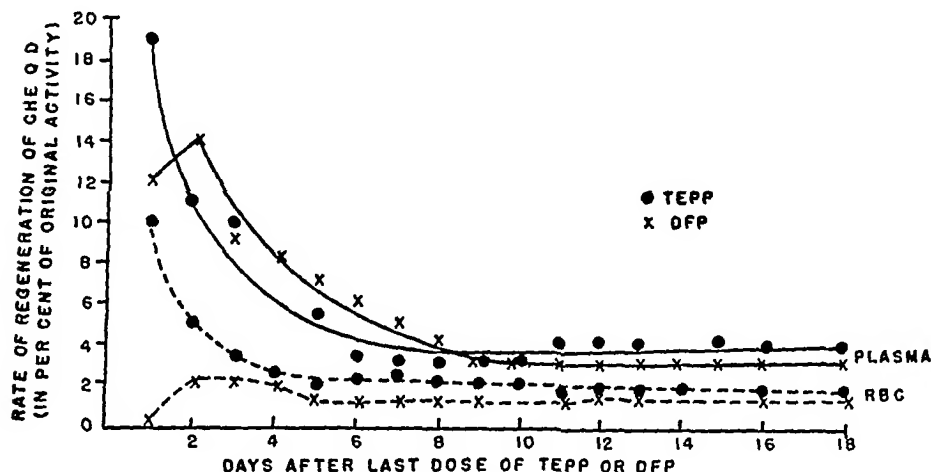


FIG 5 Comparison between the rate of recovery of plasma and red blood cell cholinesterase activity after depression by TEPP and by DFP administered intramuscularly (Average values obtained in 14 subjects (TEPP) and 35 subjects (DFP). The vehicle (water or peanut oil) did not influence the rate of recovery.)

a first-order reaction and with predominantly irreversible inactivation of the ChE within the period of observation.

#### *The Rate of Return of Plasma and Red Blood Cell Cholinesterase Activity after Cessation of Administration of TEPP*

The maximum depression of plasma ChE activity occurred within one hour after the administration of TEPP by any route (Fig 2). Following this period there was a gradual return of the plasma ChE activity at an average rate of 19 per cent of the original level during the first 24 hours and 11 per cent during the second 24 hours (Figs 4 and 5). This rate of return gradually fell off to about 4 per cent per day by the sixth day, and 3 per cent by the tenth day. The rate of return of

plasma ChE activity after depression by TEPP was somewhat more rapid during the first 24 hours than after depression by DFP, but following this period the rates of return were approximately the same (Fig 5)

The maximum depression of red blood cell ChE activity occurred within two hours after the administration of TEPP by any route. The return of this ChE activity was also most rapid during the first 24 hours, increasing at an average rate of 10 per cent of original activity (Figs 4 and 5). The rate of return diminished to 5 per cent during the second day and to slightly less than 2 per cent by the fourth day, at which rate it remained until normal levels were reached. This rate of recovery of ChE activity in the red cells after depression by TEPP was significantly more rapid during the first 24 to 48 hours than after the administration of DFP. After this time it was only slightly greater than that seen following DFP (Fig 5).

As was the case with DFP the rate of return of plasma and red blood cell ChE activity after the administration of TEPP was independent of the dose of drug, of the time over which it was administered, and of the existing ChE activity. Recovery of ChE activity was always complete, and there was no difference found between normal and myasthenic subjects.

#### *Symptoms Following the Administration of TEPP to Human Subjects*

The dosage necessary for the production of symptoms due to TEPP administered parenterally (in aqueous solution or in peanut oil) was 5 mg in one injection, 3.6 mg daily for 2 days, or 2.4 mg daily for 3 days. Similar results were obtained following the oral administration (in propylene glycol) of 7.2 mg every 3 hours for 3 to 5 doses.

The symptoms that followed the administration of TEPP to human subjects qualitatively resembled those which followed DFP, and included most of the muscarinic and nicotinic effects of cholinergic drugs (except for the absence of any significant peripheral vasodilatation or fall in blood pressure), as well as symptoms referable to the central nervous system (Table 5). However, there were certain very striking quantitative differences between the symptoms produced by TEPP and those which followed DFP.

The nicotinic effects of TEPP were much more striking than those

TABLE 5

*Signs and Symptoms that Followed the Administration of TEPP to 16 Subjects (4 Non-myasthenic and 12 Myasthenic) in Doses Described Above, Without Atropine, and in Doses Described below for the Treatment of Myasthenia Gravis, with the Simultaneous Administration of Atropine*

<i>Effector organ</i>		<i>Number of subjects</i>
<b>1 Muscarinic</b>		
a) Gastro-intestinal	anorexia, nausea	15
	vomiting	10
	abdominal cramps (mild)	8
	cardiospasm	5
	diarrhea	5
	eructation, "heartburn"	5
b) Sweat glands	increased sweating	13
c) Salivary glands	increased salivation	6
d) Lacrimal glands	increased lacrimation	2
e) Heart	bradycardia	3
	prolongation of PR and QRS intervals	1
f) Peripheral vascular	subjective warmth, "hot flashes"	4
g) Lungs	respiratory difficulty	4
h) Pupils	miosis	3
i) Ciliary body	difficulty of distant vision	3
j) Bladder	frequency (mild)	3
<b>2 Nicotinic</b>		
	local fasciculations at site of 1 m injection, in non-myasthenics	4
	generalized fasciculation, in non-myasthenics	1
	myasthenics	9
	increased strength, in myasthenics	12
	weakness (following overdose), in myasthenics	2
	weakness (slight), in non-myasthenics	3
<b>3 Central Nervous System</b>		
	giddiness	15
	uneasiness, restlessness, tremulousness	12
	insomnia	9
	headache	8
	paresthesias	6
	excess dreaming, occ nightmares	6
	EEG change (slow waves)	3
	drowsiness	2
	tremor	2
	mental confusion	2
	emotional lability, with depression	2

of DFP When TEPP was injected intramuscularly localized fasciculations appeared in normal subjects, and with higher doses there was slight weakness accompanied by generalized muscular fasciculations These were most marked in the facial and extra-ocular muscles, sometimes causing uncontrollable jerking movements of the eyes and tremor of the eyelids In myasthenic subjects there was a pronounced increase in strength and fasciculations were frequently noted, especially in those muscles least involved in the myasthenic process

The muscarinic effects of TEPP, which were the first to appear, included anorexia, nausea, vomiting, and a sense of pressure beneath the lower sternum suggestive of cardiospasm Abdominal cramps were mild and fleeting, and diarrhea was observed chiefly when neostigmine was administered subsequent to the TEPP Profuse sweating and excessive salivation were common Lacrimation, miosis, difficulty in distant vision, bronchoconstriction, urinary frequency and bradycardia occasionally appeared after larger doses of the drug

The central nervous system symptoms which followed the administration of TEPP consisted chiefly of uneasiness, restlessness, tremulousness, and giddiness Larger doses of drug sometimes caused headache and paresthesias, and following prolonged administration there was occasionally drowsiness, tremor, mental confusion, and emotional lability, with depression Insomnia, excessive dreaming, and nightmares, which were common after DFP, were less frequent and less severe following TEPP The paresthesias accompanied only the larger doses of the drug and were variously described as a feeling of "tingling," "pins and needles," "warm waves", and "electric shocks" These were usually intermittent and generalized, often beginning in the facial region and spreading over the body and limbs Electroencephalographic changes following TEPP were minimal and much less frequent than after the administration of DFP Three patients did develop occasional slow waves in their electroencephalograms, occurring at a rate of 3 to 6 per second and similar to the more striking bursts of slow waves seen following DFP

When symptoms appeared they usually began suddenly about 30 minutes after the last dose of the drug Muscular fasciculations were the most transient, almost always disappearing within one hour after onset Vomiting was also of short duration The remaining musca-

rinic manifestations, as well as the central nervous system symptoms, usually persisted for periods ranging from 1 to 5 hours. After large doses of TEPP nausea and giddiness were present for as long as 24 hours, and it was quite common for patients to feel comfortable while in the recumbent position after the overt symptoms had worn off, only to have a return of giddiness if they sat up or walked about. This was not accompanied by any change in the blood pressure. Increase in strength in the patients with myasthenia gravis persisted for a period of 12 to 72 hours. Local instillation of TEPP in peanut oil in a concentration of 0.5 mg per ml resulted in marked miosis lasting for 12 to 48 hours.

After the administration of TEPP symptoms appeared more abruptly, were more explosive in nature, and also disappeared more rapidly than after DFP. Repeated administration of TEPP, like DFP, resulted in an increasing symptomatic effect of each dose, presumably because of the progressive inhibition of ChE. However, the cumulative effect of repeated doses was not nearly as striking as in the case of DFP, where the potentiation of subsequent doses was seen over a much longer period of time. Potentiation of repeated doses of TEPP was striking within several hours of a given dose but diminished rather rapidly during the subsequent few days, whereas the sensitizing effect of a dose of DFP could be demonstrated for a period of 3 to 4 weeks. (3) This difference is presumed to be due to a more rapid return of ChE activity in the tissues after depression by TEPP than by DFP.

The ChE activity of the plasma was reduced to nearly zero before the appearance of symptoms due to TEPP. When TEPP was administered over a relatively short period of time, in doses described above, symptoms appeared at red blood cell ChE levels between 13 and 25 per cent of the original activity. This appearance of symptoms at a particular level of red blood cell ChE activity is probably due to the similarity in the sensitivity of the ChE enzymes of the red blood cells, muscle, and brain to TEPP, as demonstrated *in vitro*. However, as in the case of DFP, there was no constant relation between the presence of symptoms and the degree of depression of red blood cell ChE activity, probably because of the slower rate of restoration of red blood cell ChE, compared to the tissue cholinesterases. The ChE activity of the red cells could be depressed to very low levels (8 per cent) by TEPP without the occurrence of any symptoms by the daily admin-

istration of small doses (0.5 to 1 mg daily intramuscularly) When symptoms did occur following the administration of TEPP they disappeared while the red blood cell ChE was at a low level of activity, being frequently less than 15 per cent of the original activity After several weeks of daily administration of TEPP in the treatment of myasthenia gravis, the plasma and red blood cell cholinesterases were depressed to, or near, zero activity, so that the activity of these enzymes could not be used as a guide in the regulation of the dosage schedule

The administration of atropine by any route had a moderate to marked inhibitory effect on the muscarinic symptoms due to TEPP, a slightly inhibitory effect on the central nervous system symptoms, and no effect on the nicotinic manifestations If the muscarinic effects were moderately severe, 2 to 3 mg of atropine intramuscularly were required every hour as long as symptoms persisted in order to provide relief It was quite evident that in this situation the tolerance for atropine was increased to many times the dose of this drug ordinarily producing signs of over-atropinization The central nervous system symptoms due to TEPP could be further reduced by the administration of phenobarbital

#### *The Effect of TEPP on the Gastro-Intestinal Tract*

The gastro-intestinal symptoms that followed the administration of TEPP to both normal and myasthenic subjects were less marked and more transient than was the case with DFP The motility of the small and large intestine, studied by intubation (3), was not as strikingly affected by TEPP as by DFP There was only a very slight effect from a single, relatively large dose of TEPP (3 mg in oil intramuscularly) A moderate effect upon gastro-intestinal motility appeared after the second and third doses when these were given daily but persisted for only one-half to two hours, in contrast to three to five hours after DFP (3) The prior administration of TEPP sensitized the intestinal tract to the stimulating effect of neostigmine and pitressin, but this sensitization did not persist for as long a period of time as had been the case with DFP The antagonistic effect of atropine on the smooth muscle contractions due to TEPP was the same as on those due to DFP

DFP administration results in the development of rhythmical con-



tractions of the intestines lasting for long periods of time, and the sensitizing effect which it has upon subthreshold doses of neostigmine and pitressin persists for one to two weeks (3). Thus, it has been a more satisfactory drug for the treatment of paralytic ileus than TEPP.

*The Effect of TEPP on Neuromuscular Function in Normal Subjects and in Myasthenia Gravis*

The effects of TEPP on neuromuscular function were in general similar to those of DFP and neostigmine, though resembling neostigmine more closely. The duration of the effects of TEPP was shorter than those of DFP and longer than those of neostigmine.

The intramuscular administration of 0.5 to 5 mg of TEPP to normal subjects invariably resulted in the immediate development of fasciculations in the injected muscle. These were extremely numerous and lasted for from one to twenty minutes, being more numerous and less prolonged when the TEPP was in aqueous solution than in peanut oil. When TEPP was administered daily the duration of the local fasciculations following each injection decreased progressively from about ten minutes on the first day to one minute on the sixth. When the doses were high, generalized fasciculations soon appeared in the normal subjects, and there was often a mild generalized weakness, with increased fatigability after activity.

TEPP was injected into the brachial artery of eleven normal subjects, in amounts from 0.25 to 2.5 mg in aqueous solution. The administration of 0.5 mg or more of this drug by the intra-arterial route resulted immediately in the appearance of myriads of fasciculations, and moderate to marked weakness, in the injected forearm and hand. The degree and duration of the fasciculations and weakness were proportional to the dose administered. The fasciculations were maximal immediately after the injection and gradually disappeared over a period of one to two hours. A similar time course was recorded for the muscular weakness, although the return to completely normal strength was somewhat more delayed (Fig. 6). Sweating usually appeared over the injected forearm and hand despite the previous administration of 0.6 to 1.2 mg of atropine intramuscularly. The duration of changes in function which followed the intra-arterial administration of TEPP was more than twice that of the similar effects following an

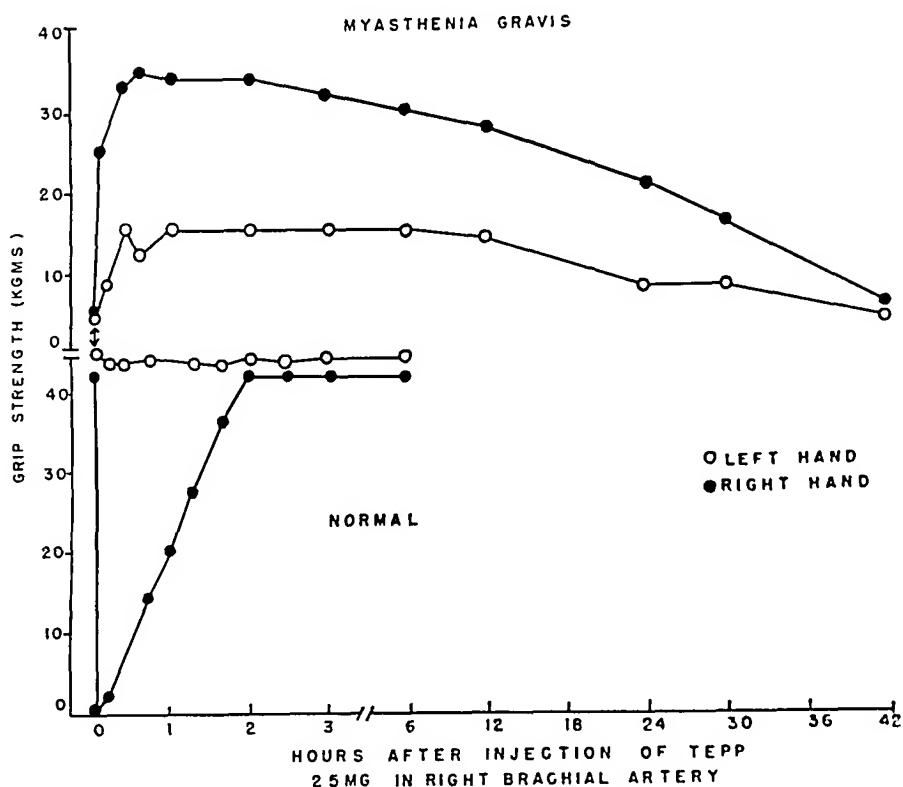


FIG 6 The effect of TEPP administered intra-arterially on muscle strength in a normal subject (C D) and in a patient with myasthenia gravis (G R)

TABLE 6

*Duration of the Effect of Anticholinesterase Agents on Muscular Strength after Intra arterial Administration*

(Hours)

DOSE	NORMAL SUBJECTS		MYASTHENIC SUBJECTS
	Inasculations	Weakness	Increased strength
Neostigmine (0.5-3.0 mg)	0.1-0.5	0.3-0.7	3-5
TEPP (1.0-2.5 mg)	0.5-1.0	1-2	24-48
DFP (0.5-2.0 mg)	0.5-216	24-1920	24-216

equal dose of neostigmine, but much less than the duration of such changes after the injection of DFP (Table 6)

Electromyographic studies showed that the effects of TEPP, DFP,

and neostigmine on neuromuscular function in normal subjects are similar in most respects, the most striking difference being that of the duration of the observed alterations. Following the intra-arterial injection of TEPP there was no change in the voltage or duration of the muscle action potential produced by a single maximal motor nerve stimulus, but the normal diphasic potential was converted into a re-

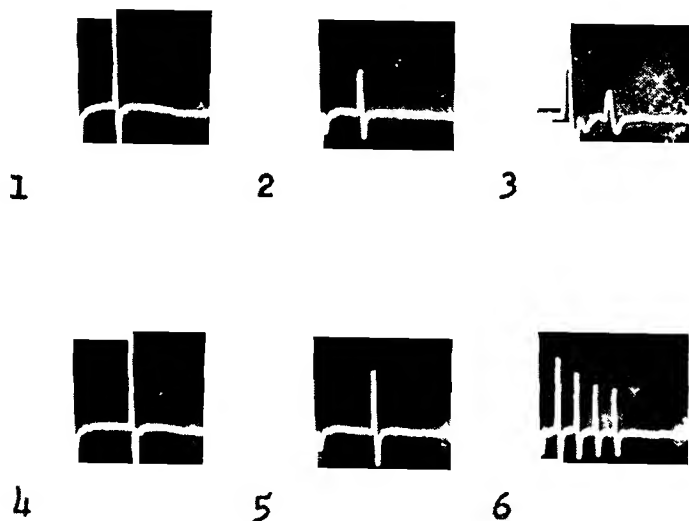


FIG 7 The effect of TEPP on the electromyogram of a normal subject (C D) 1 and 4 Responses to 2 nerve stimuli, at intervals of 45 and 60 msec, before TEPP 2 and 5 Responses to similar stimuli 15 min after the injection of 2 mg TEPP into the brachial artery, showing the depressed voltage of the second response, which is more marked when the interval between the stimuli is short 3 Faster sweep speed showing the repetitive character of the initial response after TEPP 6 Response to a train of 5 stimuli after TEPP, showing the progressive decline in voltage of successive potentials Initial response in 1 equals 8 mV

petitive response consisting of a series of spikes diminishing progressively in voltage and duration. When two maximal motor nerve stimuli were applied at varying intervals of time there was no repetitive response after the second stimulus unless the interval between the two stimuli exceeded 150 milliseconds. There was a very significant reduction in the voltage of the response to the second stimulus, the reduction being greatest at the shortest interval between the two stimuli (Fig 7). The muscle action potential responses to a train of

motor nerve stimuli at rates varying from 12 to 50 per second also showed a reduction in the response to successive stimuli. These potentials declined progressively in the same fashion as those observed after the administration of neostigmine.

The intra-arterial injection of d-tubocurarine chloride a few minutes after the administration of TEPP resulted in a prompt disappearance of the fasciculations and a more rapid rate of return of muscular strength than had occurred previously following the injection of TEPP alone into the opposite brachial artery.

TEPP was injected into the brachial artery of three patients with myasthenia gravis. In all of these patients there was moderate to severe weakness of the muscles of the upper extremities in the basal state. At the time of the injection no neostigmine had been administered for at least 6 hours. The injection of 2.5 mg of TEPP resulted in a prompt increase in the strength of the injected forearm and hand reaching a maximum within 10 minutes (Fig. 6). There was slight sweating below the level of the injection despite the prior intramuscular injection of 0.6 mg of atropine. No fasciculations were observed in the injected arm or elsewhere. After the release of the pressure cuff which had been applied above the site of injection, there was a slight, but definite increase in strength of the opposite arm and also in the affected muscles generally (Fig. 6). The local increase in strength of the injected arm remained at a maximal level for a period of 6 hours and then declined slowly, reaching in about 40 hours the basal level of weakness which existed before injection. The slight increase in strength of the muscles elsewhere declined progressively over a period of 20 hours.

Electromyographic studies were carried out in all three myasthenic patients. Before the injection of TEPP the muscle action potentials in response to stimulation of the ulnar nerve revealed in each instance the characteristic defect in neuromuscular conduction which has been described in this disease (20). There was a progressive decline in the amplitude of the muscle action potentials elicited by a train of nerve impulses applied at a frequency of from ten to fifty per second. Following the intra-arterial injection of TEPP this defect in neuromuscular conduction was repaired and the action potentials in response to trains of nerve impulses, of the frequency mentioned, all had the same voltage (Fig. 8). In one patient with severe myasthenia gravis there

was a partial block in neuromuscular conduction before the administration of TEPP, with a reduced voltage of the action potential in response to a single nerve volley. After the injection of TEPP the voltage of the single response was increased by 25 per cent. In all instances records taken 48 hours after the intra-arterial injection of

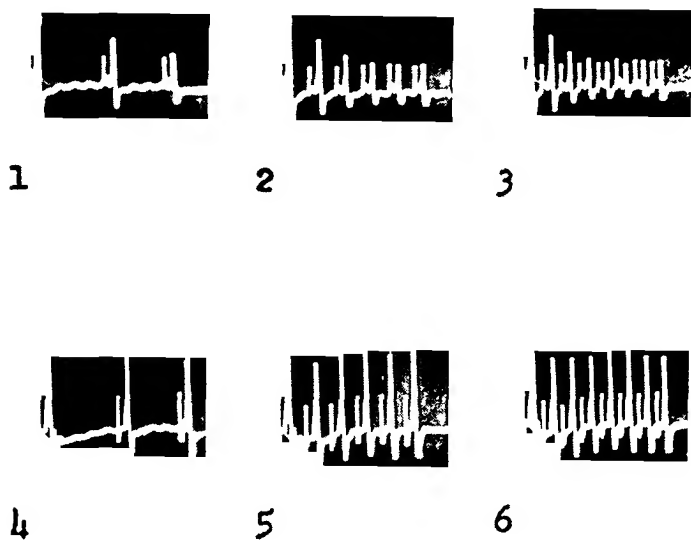


FIG. 8 The effect of TEPP on the electromyogram of a patient with myasthenia gravis (G. R.). 1, 2, and 3 Responses to trains of 3, 6, and 8 stimuli (30, 60, and 80 per sec.) before TEPP, showing the characteristic progressive decrease in voltage of successive potentials. 4, 5, and 6 Responses to similar stimuli 15 min after the injection of 2.5 mg TEPP into the brachial artery. Initial response in 1 equals 6 mV.

TEPP showed that the electromyograms had returned almost completely to the pre-injection pattern.

The effect of TEPP on neuromuscular function of myasthenic subjects following intra-arterial administration was intermediate between that of DFP and neostigmine with respect to both duration of action and degree of localization, the effect of DFP being most prolonged (Table 6) and most localized to the injected extremity.

*Blocking of the Action of TEPP by the Prior Administration of Neostigmine*

The administration of neostigmine shortly before TEPP blocked the effect of the latter drug upon the ChE activity of the plasma and red blood cells, as well as on the increase in muscular strength that occurred in myasthenic subjects (Table 7 and Fig 9). The similar effect of neostigmine upon the action of DFP has been demonstrated both in regard to ChE activity (18) and nicotinic effects (5). It seems probable

TABLE 7

*The Blocking Effect of Neostigmine on the Inhibition of Plasma and Red Blood Cell ChE Activity by TEPP*

SUBJECT	NEOSTIGMINE INJECTED I.M. 1 HOUR BEFORE TEPP	ChE (IN PER CENT OF ORIGINAL ACTIVITY) AT TIME OF ADMINISTRATION OF TEPP (2.5 MG I.A.)		DECREASE IN ChE 1 HOUR AFTER TEPP (IN PER CENT OF ChE ACTIVITY AT TIME OF ADMINI- STRATION OF TEPP)			
		Plasma	RBC	Observed		Expected*	
				Plasma	RBC	Plasma	RBC
C D	None	100	100	99	60	98	62
L K	None	100	100	98	59	98	62
J H	2 mg	45	85	27	15	93	60
L K†	2 mg	34	58	41	28	94	55

\* From Fig 4 (left), which records the effect on Plasma and RBC ChE activity of TEPP administered at various levels of ChE activity

† This subject had received a previous injection of TEPP four days before. At time of neostigmine injection ChE was 43% (plasma) and 63% (rbc) of original activity. One hour after TEPP ChE was 20% (plasma) and 42% (rbc) of original activity, and four hours later 16% (plasma) and 45% (rbc) of original activity.

that the combination of neostigmine with ChE renders the enzyme insusceptible to the inhibitory action of these phosphoric acid derivatives. By the time the ChE has been released from its combination with neostigmine, the free TEPP or DFP has apparently been rendered inactive, probably as a result of hydrolysis, which may be accelerated by enzymatic activity. Mazur has demonstrated a fluorophosphatase in plasma, red blood cells, and tissues which accelerates the hydrolysis of DFP (21). A pyrophosphatase has been described in red blood cells, but it is said to require the presence of added magnesium for its complete activity (22).

This blocking effect of neostigmine was important during the treatment of myasthenic patients with TEPP. Whenever supplementary doses of neostigmine were required, it was necessary to administer the TEPP at a time when the patient was not under the influence of neostigmine in order to obtain full benefit of its effect. Therefo

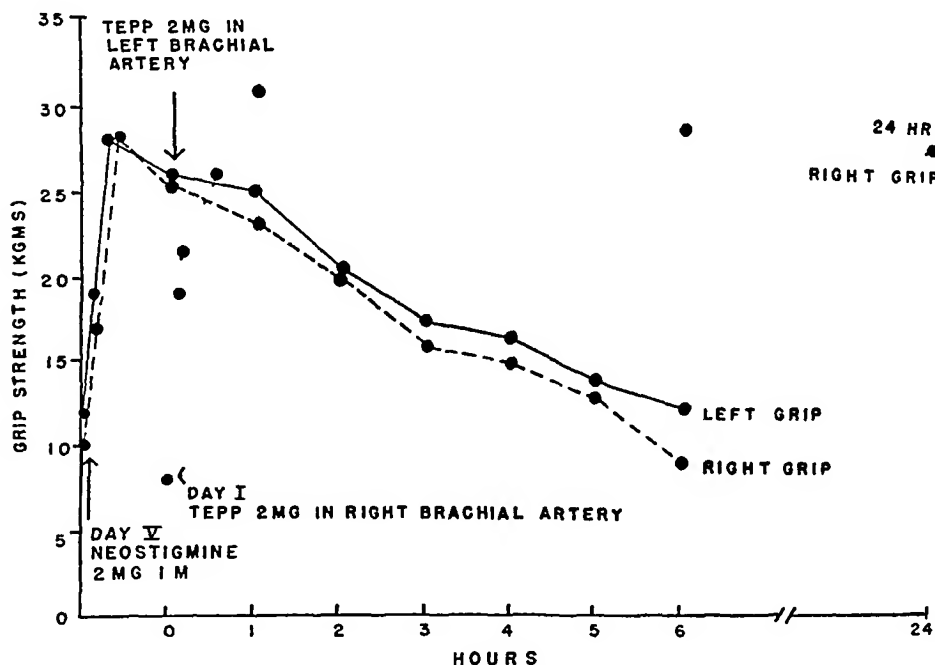


FIG 9 The blocking effect of neostigmine on the response of myasthenic muscle to TEPP injected intra-arterially. TEPP was administered intra-arterially on Day I and caused a sustained increase in the strength of the injected extremity. On Day V, when TEPP was injected intra-arterially into the opposite extremity while the patient was under the influence of previously administered neostigmine, there was no such prolonged increase in strength.

whenever possible, TEPP was not administered until four or more hours after the last dose of neostigmine.

### *The Use of TEPP in the Treatment of Patients with Myasthenia Gravis*

The intramuscular administration of 2.5 mg of TEPP (in aqueous solution or in peanut oil), or the oral administration of 10 mg of TEPP in propylene glycol, to myasthenic subjects in a basal state who had received none of the drug previously and no neostigmine for at least

six hours resulted in no increase in strength. It did cause a reduction in the ChE of the plasma and of the red blood cells to 5 and 34 per cent of original activity respectively. Repetition of the above doses of TEPP 6 to 24 hours later caused only a slight increase in general strength, and further reduction of the ChE to 3 and 25 per cent of original activity. A third dose of the drug, however, was usually followed within half an hour by a marked increase in general strength to or near the maximum obtainable after full doses of neostigmine. This increase in strength, if near maximum, was frequently accompanied by transient muscular fasciculations lasting for from several minutes to an hour. These were often most prominent in the extra-ocular muscles, resulting at times in uncontrolled jerking movements of the eyes, and in the periorbital muscles, causing tremor of the eyelids. In addition to these nicotinic effects of TEPP the patients frequently experienced symptoms due to the muscarinic and central nervous system effects despite the prior administration of 1.2 mg of atropine orally or intramuscularly with each dose of TEPP. These symptoms included anorexia and nausea, occasional vomiting, sweating, a feeling of warmth, giddiness, uneasiness, restlessness, tremulousness, and sometimes headache.

The ChE activity of the plasma and red blood cells when the muscle power approached maximal was approximately 2 and 20 per cent of original activity respectively. This was usually the level of ChE activity when muscarinic and central nervous system effects also first appeared.

In general, the maximal effects of TEPP on muscular strength were similar to those produced by an optimal dosage schedule of neostigmine in the same patient. Occasionally, muscles which responded only partially to such doses of neostigmine showed a slightly greater degree of improvement after the largest tolerated doses of TEPP. The most striking difference between the two drugs was in the duration of the increased muscular strength, which remained maximal for 6 to 12 hours after the administration of TEPP, and then gradually declined over a period of 48 to 72 hours (Fig. 10), in contrast to maximal strength for 1 or 2 hours after neostigmine, declining over 3 or 4 hours. The doses of TEPP required to maintain maximum strength once this was attained were considerably less than the initial doses required to reach the full degree of improvement.



Twelve subjects with moderately severe and very severe myasthenia gravis, who had previously been maintained on neostigmine, have been treated with TEPP over periods up to eight months (Tables 8 and 9). The most convenient method of beginning treatment was to administer the drug orally every hour in doses of about 5 mg, with atropine, until the patient reached maximum strength. The average amount of TEPP required was 41 mg administered orally over a period of five to twenty-

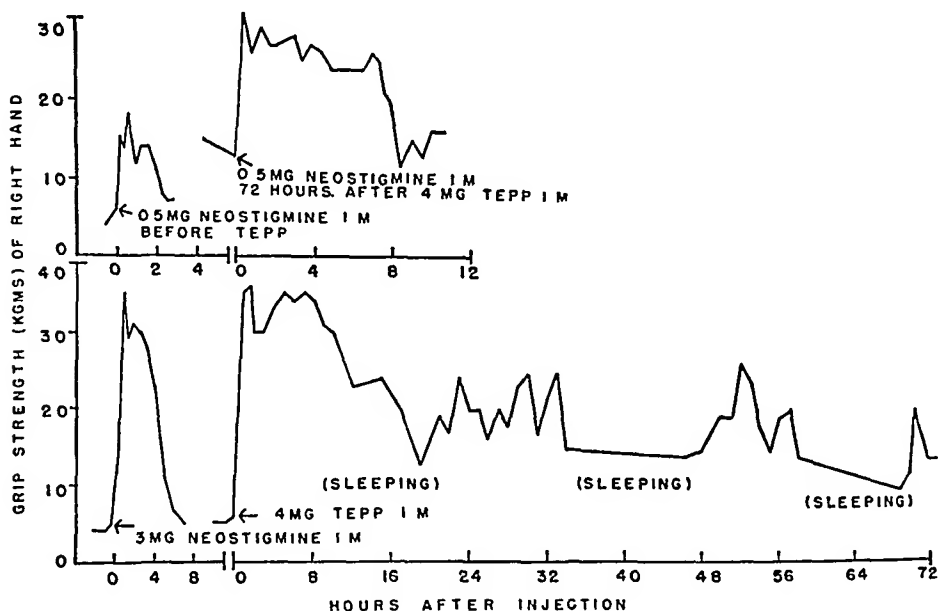


FIG 10 *Below* Comparison between the effect on muscle strength of neostigmine and TEPP administered intramuscularly to a patient with myasthenia gravis (G R). The patient had received 2 mg TEPP i.m. the day before the injection of 4 mg TEPP, with no appreciable response.

*Above* Potentiation of the effect of neostigmine on muscle strength by the previous administration of TEPP.

nine hours, or 7.2 mg administered intramuscularly over two to forty-eight hours. The average daily requirement of TEPP for maintenance of strength was 16 mg orally, or 4.8 mg intramuscularly, divided into two or three doses, the morning dose being the largest. The required initial and maintenance doses of TEPP were in general proportional to the severity of the myasthenia gravis and to the neostigmine requirement before the administration of TEPP. Atropine was administered in doses up to 10 mg orally per day in order to reduce the muscarinic and central nervous system effects of the TEPP to a minimum.

The differences between the dosage level of TEPP which produced maximum strength with a minimum of side effects, the dosage level which had little effect on motor power, and the level which caused prohibitive side-effects, were very narrow. Reduction in the daily maintenance dose of the drug by 3 to 4 mg (orally) was followed by a reduction in general strength to less than half of the maximum, while increasing the daily dose by 2 to 3 mg resulted in the appearance of troublesome muscarinic and central nervous system symptoms which could not be controlled fully by large doses of atropine (up to 10 mg orally and 8 mg intramuscularly over 24 hours). TEPP administration at this increased level also produced uncomfortable muscular fasciculations. In one patient an excessive dose of the drug administered intramuscularly resulted in transient symptoms suggestive of laryngeal spasm, possibly due to the occurrence of muscular fasciculations in this region. In two patients (H L and A R) the continued administration at home of excessive doses of TEPP in spite of obvious symptoms of overdose resulted in muscular weakness, as well as fasciculations, beginning in the muscles of the face, tongue, jaws, and pharynx and becoming generalized. In these patients the administration of TEPP was promptly stopped, following which the fasciculations ceased and the muscular strength gradually increased over a period of 48 to 72 hours to the maximal level that had existed previously.

The occurrence of muscular weakness and fasciculations in these two myasthenic patients following the continued administration of excessive amounts of TEPP resembles the effects of anticholinesterase agents in normal subjects. Similar effects were not observed in the other myasthenic subjects, even though most of them received excessive amounts of TEPP at some time during their regulation.

The narrowness of the optimal range of TEPP dosage necessitated careful preliminary adjustment of the treatment schedule and observation of the effect of the maintenance dosage for at least two weeks prior to discharge of the patients from the hospital. The occurrence of cumulative effects during the first week of administration of the maintenance dose frequently necessitated reduction of the dose during this time. A further reduction in the maintenance dose was sometimes possible after several weeks of daily administration of the drug. It proved of great importance that patients and their families be carefully instructed in the signs and symptoms of overdosage of TEPP, and in

TABLE 8  
*Description of Cases of Myasthenia Gravis Treated with TEPP*

PT	AGE	SEX	COLOR	WT kg	SEVERITY OF WEAKNESS		DURATION yrs	THYMECTOMY	HISTORY OF REMISSION	NEOSTIGMINE REQUIREMENT (mg q d)		ATROPINE REQUIREMENT mg q d (o)	DURATION OF NEOSTIGMINE RX yrs	DESCRIPTION OF BASAL STATE*
					Oculo bulbar	Per ipheral				(o)	(type)			
L K	30	F	C	38	++	++	13	7 yrs ago	Partial after thy-mec-tomy	75	0	0	12	Facial muscles weak, up and about, but with easy fatigue of arms and legs
M W	41	F	W	57	++	++	10	7 yrs ago	Partial after thy-mec-tomy	90	0	0	9	Facial and masticatory muscles weak, unable to sit up, could barely raise arms
H L	52	F	W	61	+++	+++	2	1½ yr ago	0	90	0	0 6	2	Unable to swallow semi-solids, chew, smile, speak intelligibly, raise head, or sit up
R N	30	F	W	48	++++	++	8	0	0	120	0 5	0 6	8	Unable to swallow liquids, voice unintelligible, facial muscles, arms and shoulders very weak
D L	19	F	W	48	++++	++++	3	0	0	120	0	0	3	Could barely swallow liquids and raise arms, unable to smile or sit up

G R	30	F	W	53	+++	+++	+++	8	0	0	180	0	1	2	3	Could swallow liquids only, unable to raise arms or legs, sit up, or roll over in bed
G K	60	M	W	75	+	+++	+++	4	0	0	135	0	1	2	2	Could barely swallow liquids, chew, smile, or get out of bed, speech unintelligible
F W	29	F	W	48	+++	+++	+++	4	1 yr ago	0	150	0	5	1	3	Unable to swallow liquids, speak intelligibly, smile, raise arms, or sit up
A M	33	M	C	66	+	+++	+++	4	3 yrs ago	0	180	0	0	0	3	Unable to raise head, arms, or sit up
A R	45	F	W	53	+++	+++	+++	14	0	0	225	0	1	6	10	Unable to swallow semi-solids, chew, smile, speak intelligibly, raise head, or sit up
S D	38	F	W	68	+++	+++	+++	5	0	0	300	0	0	0	5	Unable to swallow semi-solids, chew, smile, raise arms, or sit up
J H	30	M	W	61	+++	+++	+++	3	1 yr ago	0	540	1	0	9	3	Unable to swallow liquids, speak intelligibly, smile, raise arms, or sit up
																Poor response to neostigmine

\* All patients also had ptosis and limitation of extra-ocular movements, with diplopia, in the basal state

TABLE 9

*The TEPP Requirement of 12 Myasthenic Patients, and the Effect of TEPP on Grip Strength, on Atropine Requirement, and on Muscles Which did not Respond Fully to Neostigmine*

TEPP was administered orally in propylene glycol in 2 or 3 divided doses daily, or (to G R and J H) intramuscularly in peanut oil. Grip strength is of the right hand

BEFORE TEPP			DURING TEPP					ATROPINE REQUIREMENT	MUSCLES THAT DID NOT REACH FULL STRENGTH AFTER NEOSTIGMINE OR TEPP
Grip Strength (Kg)			TEPP Requirement (mg (o) q d)						
Basal	Max after Neostig	Basal	Max	Initial*	Half Max Strength	Maintenance			
				hrs		Max Strength	Max Strength and Marked Side Effects	mg (o) q d	
L K	13	31	27	32	40 (29)	8	12	15	EOM
M W	2	25	24	27	22 (5)	13	16	18	EOM
H L	6	25	23	28	30 (6)	13	16	18†	EOM
R N	7	26	21	29	26 (8)	8	11	13	EOM, facial, speech, extensors of forearms, hands, fingers
D L	6	25	24	33	46 (28)	11	14	16	EOM, extremities
G R	3	35	30	39	46 (13) or 1 m	12	18	20	EOM
G K	16	29	31	33	7 2 (48)	13	16	19	EOM, speech, legs
F W	1	25	18	27	36 (24)	13	16	18	EOM, extremities
A M	7	45	40	50	60 (72)	17	20	22	None
A R	1	15	15	26	50 (12)	14	17	19†	EOM, extremities
S D	9	26	23	30	45 (8)	12	14	16	EOM, facial
J H	6	19	17	20	7 1 m (2)	12†	18†	21†	All muscles†
Average	6	27	24	31	41 (20)	12	16	18	7

\* Amount of TEPP required to increase the basal strength when administered over the indicated time

† Patient J H, a very severe myasthenic who responded poorly to large doses of neostigmine, was the only one who could not be regulated on TEPP alone, requiring, in addition, 240 mg (o) of neostigmine daily to maintain respiration

‡ These doses of TEPP administered to patients H L and A R resulted in generalized muscular weakness, in addition to marked side effects. TEPP was discontinued in these two patients and neostigmine resumed

the importance of adjusting the dose in accordance with the response of the patient

When the drug was discontinued the patients' strength declined gradually to the original basal state in about 48 hours. If the drug was resumed at this point considerably more than the maintenance dose had to be given to regain maximal strength, although not as much as was originally required. There was no change in the severity of the myasthenia gravis or in its response to neostigmine after cessation of treatment with TEPP.

When TEPP was administered in amounts sufficient to increase the muscular strength of patients with myasthenia gravis above the basal level but not to a maximum there was usually a significant potentiation of the effect of subsequent doses of neostigmine administered either intramuscularly or orally. The degree of improvement in muscular power after small doses of neostigmine was in most cases significantly increased and the effect lasted two or three times longer than before the administration of TEPP (Fig 10). This potentiation of neostigmine action could be demonstrated for only about forty-eight hours after TEPP administration was discontinued, at which time there was still a pronounced reduction in the ChE activity of both red cells and plasma. On the other hand, when sufficient TEPP had been administered to bring about a maximum increase in muscular strength, the administration of neostigmine resulted in no further improvement, even in muscles which had not responded fully to the administration of TEPP. In three patients with severe myasthenia gravis (R N, F W, and A R) the administration of 1 to 2 mg of neostigmine intramuscularly at a time when the patients were at maximum strength following TEPP produced a generalized weakness which was very striking. There was difficulty in swallowing, impairment of speech, sagging of the jaw, marked ptosis, and severe weakness in the extremities. In two patients there was a gradual return of strength over a period of four hours after the administration of neostigmine.

In the third patient (A R) failure to recognize the weakness as being due to excess of anticholinesterase drug nearly led to a fatal outcome. This patient was regulated on 18.5 mg of TEPP (orally) daily, and 10 mg of atropine. On this schedule her general strength and endurance were better than when she had been taking neostigmine, and she had no

symptoms suggestive of overdose of TEPP during the week that she was in the hospital. Two days after discharge, however, she developed nausea, sweating, uneasiness, and weakness of the muscles of the face, tongue, and pharynx, accompanied by fasciculations. The patient took an additional 2 mg of TEPP in the belief that this would increase her strength, and she became still weaker. Although she had been advised against taking neostigmine while on her full TEPP schedule she then took 30 mg of neostigmine by mouth. This was followed by generalized weakness and muscular fasciculations, with inability to swallow or speak. She was rushed to the emergency room where the physician on call administered 1 mg of neostigmine intramuscularly. This was followed by sweating, salivation, generalized muscular fasciculations, profound generalized weakness, and finally cessation of respiration with loss of consciousness. An endotracheal catheter was inserted, and artificial respiration and oxygen administered. The patient was placed in a Drinker respirator, and soon regained consciousness. She was given no medication during the next 24 hours, except atropine intramuscularly. Her strength gradually increased to the maximal level that had existed previously, and the muscular fasciculations disappeared.

Of the twelve myasthenic patients who have been treated with TEPP, neostigmine has been withdrawn during the time of administration of TEPP in all except one patient (J H), who had critically severe myasthenia gravis and required enormous doses of neostigmine. TEPP had to be discontinued in two patients (H L and A R) who developed muscular weakness following the administration of excessive amounts of this drug, and neostigmine was resumed. The remaining nine patients were maintained on TEPP for periods of one to eight months, and all had better general strength and endurance than during the administration of neostigmine. Three of the nine patients (R N, D L, and S D) had moderately severe nausea due to TEPP which was not entirely prevented by atropine. After being maintained on TEPP for periods of one to two months they requested that they be returned to neostigmine, in spite of the increased strength which the TEPP had afforded. The remaining six patients have been satisfactorily maintained on TEPP for four to eight months and prefer this drug to neostigmine. They are continuing to receive TEPP at the present time.

*Effects of TEPP Other Than Those Attributable to its Anti-Cholinesterase Activity*

The twelve patients with myasthenia gravis who received TEPP daily for periods up to eight months were observed carefully and no effects of the drug were noted other than those attributable to its anticholinesterase activity. There was no change in temperature or body weight. No alterations were noted in the hematocrit, hemoglobin, red cell count, white blood count or differential formula. Determinations were made at intervals of blood non-protein nitrogen, fasting blood sugar, chloride, carbon dioxide combining power, calcium, phosphorus, alkaline phosphatase, serum proteins, and bilirubin, but no changes were detected. The following tests of hepatic and renal function remained normal: thymol turbidity, cephalin flocculation, bromsulfalein retention, phenolsulphonphthalein excretion and urinalysis. The basal metabolic rate remained unchanged and teleoroentgenograms were normal. In one patient (G R) there was a slight transient prolongation of the PR and QRS intervals of the electrocardiogram.

#### DISCUSSION

Dubois and Mangun (14) were the first to point out that the insecticide, hexaethyl tetraphosphate (HETP), which had been known to have strong nicotinic effects, has a potent inhibitory effect on mammalian as well as insect ChE both *in vitro* and *in vivo*. Westerberg and Luross (22a) found that the administration of HETP to patients with myasthenia gravis resulted in an increase in strength. Mangun and Cleveland (17) demonstrated that HETP is really a mixture containing neutral esters which account for the major portion of its biologic activity, and that one active neutral ester is TEPP. Of the compounds of this group of phosphonic acid derivatives which appear to derive their activity from the unstable pyrophosphate type of linkage, TEPP seems to be the best tool for the continuation of studies on various anticholinesterase agents in the human subject.

Brauer (23) described experiments which led him to conclude that a stable compound was formed between ChE and a phosphorus-containing moiety of HETP or TEPP. While these studies *in vitro* were said to demonstrate irreversibility of the inhibition of plasma ChE under the conditions present (23), other experiments *in vivo* showed that the



brain ChE activity of rats recovered several times more rapidly after the administration of TEPP than after comparable doses of DFP (24). The experiments described in the present communication demonstrate a type of recovery curve of red cell ChE *in vivo* compatible with partial dissociation of the TEPP-ChE complex during the early period following administration of the inhibitor. After twenty-four to forty-eight hours the recovery curve is slow and at a rate compatible with the formation of new red blood cells, suggesting that at this time the return of ChE activity is by the formation of new enzyme and not by reactivation of inactivated enzyme. These observations pointing to a limited dissociation of the TEPP-ChE complex *in vivo* during the early period after formation of the complex have been supported by experiments *in vitro*, and serve to explain the shorter duration of the clinical symptoms produced by TEPP as compared to DFP. Since the red cell ChE is still greatly depressed when symptoms due to TEPP disappear, it is probable that the tissue ChE activity returns more rapidly after depression by TEPP than does that of the red blood cells, and that a speedy partial return in the first few hours might raise the level of tissue ChE to a range compatible with normal function, even though return to completely normal activity might be delayed for several days.

This partial dissociation of the inhibitor-enzyme complex would also afford an explanation of the less striking potentiation of repeated doses of TEPP in human subjects as compared to DFP. With TEPP the sensitizing effect of a given dose declines rapidly over a few days, in contrast to a period of weeks following DFP. This has enabled the daily administration of TEPP in larger doses than is possible with DFP.

The results of TEPP administration support the hypothesis previously set forth (5) that the distribution of the physiological activity of these ChE inhibitors depends to a certain degree on their physical properties. The lipid-water partitions of DFP, TEPP, and neostigmine, as demonstrated in the experiment employing peanut oil, suggested that the effects of TEPP would be likely to resemble qualitatively those of neostigmine more closely than those of DFP. Such has been the case in the studies on human subjects, and the observations make it probable that this anti-ChE agent will be useful in the treatment of myasthenia gravis.

It may be pointed out that TEPP, like DFP, has no direct excitatory

effect on muscle, in contrast to the direct effect of neostigmine described by Riker and Wescoe (25). The ability of TEPP to restore power to myasthenic muscle bears a general resemblance to the effects of neostigmine. This might suggest that, since TEPP exerts its effects only by virtue of its anticholinesterase activity, the effect of neostigmine on myasthenic muscle may likewise be due primarily to its anticholinesterase activity, rather than to its ability, as a weak choline ester, to stimulate muscle directly. However, certain differences between the action of TEPP and neostigmine must be noted. A large initial dose of TEPP is required before its physiological effects are produced, and the range of effective maintenance dose between that which produces no observable effect and that which causes distressing symptoms is narrow. In animals there is a very narrow range between the maximum dose of TEPP which is safely tolerated, and the dose which is invariably fatal. These characteristics of TEPP action are in contrast to those of neostigmine. TEPP would appear to exert its effects by depressing the ChE activity of the tissues below a threshold at which changes in function begin, and by maintaining this degree of depression. The difference between this threshold and the level of ChE activity below which serious and even fatal functional alterations occur, appears to be narrow. The mode of action of neostigmine, if similar, would seem to entail a qualitative difference in its effect on ChE enzymes and in the production of physiological effects.

The electromyographic observations in the normal subjects provide certain points of interest. Qualitatively they are similar to those seen after neostigmine and DFP. When two maximal nerve stimuli are applied, the muscle action potential in response to the second stimulus is greatly diminished in the presence of these ChE inhibitors. Full recovery does not take place until the stimuli are well over 250 msec apart. As the initial response is not changed in voltage this phenomenon of depression is a result of the changes associated with the passage of a single nerve impulse at the neuromuscular junction. Its time course is compatible with the accumulation and gradual dissipation of acetylcholine and favors the theory of chemical mediation at the neuromuscular junction.

Eleven patients with myasthenia gravis have now been treated with TEPP alone for periods up to eight months, and one patient has been

treated with TEPP supplemented by neostigmine. The initial regulation of TEPP dosage in the treatment of myasthenia gravis is rather difficult, due to the narrow range between the amount which produces a maximum effect on muscular function and that which causes unpleasant and potentially dangerous muscarinic, nicotinic, and central nervous system effects. The daily maintenance dose must be determined with care, for if it is much below the threshold amount for good, steady muscular power throughout the twenty-four hour period the strength drops to a minimal level. It is helpful to know that under these circumstances, if the strength is above the basal level, the response to neostigmine will usually be enhanced and prolonged. It has been found possible, following the initial regulation in the hospital, to administer maintenance doses of TEPP to patients on an out-patient basis, and six of twelve patients who have received the drug have preferred it to any other medication because of the maintenance of a steady increase in muscular power throughout the day and night.

#### SUMMARY

1 Tetraethyl pyrophosphate (TEPP) is a more potent inhibitor of human plasma, red blood cell, brain, and muscle cholinesterases in vitro than either di-isopropyl fluorophosphate (DFP) or neostigmine. TEPP has a greater affinity for plasma ChE than for the other cholinesterases, which are inhibited to a lesser, and approximately equal, degree. TEPP combines with ChE over a period of several minutes, following which ChE activity may be restored very slowly and incompletely during prolonged incubation, especially at 37°C.

2 The administration of TEPP to human subjects by any route causes a rapid fall of the ChE activity of the plasma to near zero, and a somewhat less striking depression of the ChE activity of the red blood cells, which can be reduced to near zero by repeated doses of TEPP or by a single very large dose. Approximately four times as great an oral dose is required to produce the same effect as when the drug is administered intramuscularly or intravascularly.

3 Within one to two hours after the administration of TEPP the ChE activity of the plasma and red blood cells begins to return toward normal, the former at a rate of 19 per cent of original activity during the first day and falling off to 4 per cent after the fifth day, the latter at a rate of 10 per cent during the first day and 2 per cent after the second

day The rates of return of ChE activity are more rapid following depression by TEPP than by DFP during the first twenty-four hours after administration, probably due to partial dissociation of the TEPP-ChE complex during this time

4 The muscarinic, nicotinic and central nervous system effects which follow the administration of TEPP to normal and myasthenic subjects have been described TEPP, like neostigmine, has more marked nicotinic effects after systemic administration, and less marked central nervous system effects, than does DFP This may be due to the greater solubility of TEPP and neostigmine in aqueous than in lipoid medium, and to the greater solubility of DFP in lipoid medium In general, the maximal effect of TEPP on the muscular strength of myasthenic patients is similar to that produced by optimal amounts of neostigmine, except that the effect of TEPP is much more prolonged, the increased strength being fully sustained for 6 to 12 hours and gradually declining over a period of 48 to 72 hours

5 Eleven patients with moderately severe and very severe myasthenia gravis who had previously been maintained on neostigmine have been treated with TEPP alone, and one with both TEPP and neostigmine The average amount of TEPP required to reach maximum or near maximum strength has been 41 mg administered orally over a period of five or more hours The average daily dose for maintenance of strength has been 16 mg orally in two or three divided doses The differences between the dosage level of TEPP which produces a maximum increase in strength with a minimum of side-effects, the dosage level which has very little effect, and the amount which causes prohibitive side-effects, are very narrow This necessitates careful preliminary adjustment of the treatment schedule, and careful observation

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# HISTOCHEMICAL STUDIES ON NUCLEIC ACID PHOSPHATASE<sup>1</sup>

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## I SPECIFICITY OF THE REACTION

Gomori (1) and Takamatsu (2) have developed a technique whereby the presumptive site of phosphatase activity can be demonstrated in tissue sections. This technique has been applied by many different investigators in a variety of studies. In view of the wide use of this procedure, it is surprising how little effort has so far been expended toward making this method applicable to the study of those specific phosphatases that are of prime interest to biochemists. Gomori, using fixed and paraffin embedded tissue, found that the histological pattern of presumptive enzyme activity was the same for all the phosphate substrates which he used. He concluded, therefore, that only the non-specific phosphomonoesterase survived the rigors of the histological technique. The variation in activity which he found in sections from various organs, corresponded satisfactorily to the organ distribution of phosphomonoesterase demonstrable by conventional techniques (3).

Glick (4) using adenosinetriphosphate as substrate on sections of tissue fixed in cold acetone and sectioned either frozen or after being embedded in paraffin, claimed that some of the phosphate liberating activity which occurs under these circumstances is attributable to the specific adenosinetriphosphatase (ATPase). The evidence for such specific enzyme activity, however, was merely that activity was found in some tissues in which the phosphomonoesterase is sparingly present. Moog (5) points out that the specific ATPase is 70% inhibited by exposure for one hour to cold acetone, and doubts whether appreciable activity of this enzyme could be demonstrated after 24 hours fixation.

Sullmann (6) using sections of alcohol fixed and paraffin embedded cornea finds some variation in the location of the histochemical reaction.

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product depending on the substrate which he used. His results suggest that some moiety of the various specific phosphatases may survive in the fixed tissues under special conditions. Such specific phosphatases could conceivably be distinguished from the non-specific phosphomonoesterase by their response to various activators and inhibitors, but no such demonstration of specificity has, so far, been described.

The one solid study in which the histochemical technique has been applied to a specific phosphatase system is that of Krugelis (7) who used ribose and desoxyribose nucleic acids as substrates. Most of the phosphate groups in these substances are bound in di-ester linkages and are hence inaccessible to the phosphomonoesterase enzyme.<sup>2</sup> Krugelis hoped, therefore, that activity of specific enzyme systems might be revealed in these substrates. She found that with ribose nucleic acid as substrate, the reaction product was deposited only in the cytoplasm. With desoxyribose nucleic acid (depolymerized by preliminary incubation with nuclease) the reaction product was deposited only in the nucleus, and, for cells in mitosis, only in the chromosomes. This high specificity in the location of the reaction product may be taken as strong evidence for the specificity of the enzyme systems attacking these substrates.

The present study is an attempt to extend the investigation of Krugelis by the use of frozen sections<sup>3</sup> of fresh unfixed tissue, and by the application of specific activators to the enzymatic reaction. No good reason has appeared in the literature as to why the Gomori technique should be applied exclusively to fixed tissues, nor why the investigators who have used this technique should confine their interest to those enzymes that survive in part the rigors of fixation. On the other hand, the use of activators or inhibitors might afford much

<sup>2</sup> According to Gulland (8), one quarter of the phosphate groups of yeast nucleic acid are bound by monoester linkages. Schmidt (9) reports that thymus nucleic acid in its native highly polymerized state is not attacked by the acid phosphomonoesterase obtained from prostate. After depolymerization by nuclease, he finds that one quarter of the phosphate groups are liberated by the monoesterase. The attack on this substrate was, however, quite slow even when the substrate and the enzyme were both present in high concentration.

<sup>3</sup> Frozen sections have an advantage over unsectioned fresh tissue in being more readily penetrated by substrate and reagents.



stronger evidence of enzyme specificity that has heretofore been obtained in studies of this type

#### METHOD

Fresh frozen sections of rat's kidney were incubated at 37°C for 3 to 18 hours in substrate solution containing 0.1M barbiturate buffer pH 8.5–9.5, 0.03M  $\text{CaCl}_2$ , and 0.1% substrate<sup>4</sup>. After termination of incubation, the sections were washed in distilled water, immersed for 3 minutes in a solution of cobalt nitrate, washed again and then immersed for 5 minutes in a solution of ammonium sulphide. A positive reaction consists in the deposit of black granules in the section. After a final wash the sections were mounted on slides, dehydrated and cleared. As substrates we used yeast nucleic acid (Schwartz), desoxyribose nucleic acid (prepared from rat liver by the method of Brues, Tracey and Cohn (10)), and for comparison glycerophosphate, hexose diphosphate, adenosine triphosphate (from rabbit muscle by the method of Needham (11)).

#### RESULTS

##### I *Relation of Nucleic Acid Phosphatases to Non-specific Alkaline Phosphatase*

In preliminary experiments the acid phosphatase technique<sup>2</sup> of Gomori (12) was compared with the alkaline phosphatase technique outlined above. In the acid phosphatase technique, lead ions are used to precipitate the liberated phosphate. We found that, on fresh frozen sections, the acid phosphatase technique gave unsatisfactory results in that a heavy precipitate formed in the tissue in many instances even in the absence of substrate.

With the alkaline phosphatase technique, control sections of rat's

<sup>4</sup> The histochemical reaction depends upon the precipitation of calcium phosphate at the presumptive site of splitting of inorganic phosphate from the substrate. Since calcium phosphate is not completely insoluble, some diffusion of the reaction product before precipitation is almost inevitable. If a few drops of a solution of  $\text{Na}_3\text{PO}_4$  are added to the substrate solution to the point of formation of a just perceptible cloud, and the solution is then allowed to stand at 37°C for an hour, and then is filtered at the same temperature, before addition of the frozen sections, the diffusion of the reaction product is much reduced, and the crispness of the final histological picture much enhanced.

kidney incubated without substrate were satisfactorily free of deposit, and sections incubated in the presence of glycerophosphate, hexose diphosphate, ATP, and ribose nucleic acid showed heavy deposits. Contrary to the findings of Gomori on fixed tissue, the histological pattern of the deposit in the frozen sections, unfixed before exposure to the substrate solutions, presented different patterns for each substrate presented. Desoxyribose nucleic acid presented to the tissue in its native highly viscous state failed to yield any deposit. However when the desoxyribose nucleic acid was depolymerized either by being exposed for 3 hours to boiling  $\frac{N}{10}$  HCl solution or by incubation with desoxyribose nuclease<sup>5</sup> for 24 hours at 37°C, and subsequently to boiling  $\frac{N}{10}$  HCl solution for one hour, a suitably acting substrate was obtained. Exposure to nuclease alone did not render the desoxyribose nucleic acid attackable by the tissue. Exposure of ribose nucleic acid to ribose nuclease and to boiling  $\frac{N}{10}$  HCl did not alter its characteristics as substrate for the histochemical reaction. In all the experiments that follow desoxyribose nucleic acid was used in the depolymerized condition.

Comparison of the histological distribution of deposit yielded with the two nucleic acids as substrates revealed that in the rat's kidney ribose nucleic acid is attacked both in the nuclei and in the cytoplasm, while desoxyribose nucleic acid is attacked only in the nucleus. The results of these experiments which are largely in accord with those of Kruehls indicate that the tissue distinguishes between the two nucleic acids and that, therefore, the results of the experiments can be referred to highly specific phosphatase systems.

## II *Specific Activators*

Having established the validity of the technique on frozen sections of rat's kidney, we attempted to apply the same technique to the rat's cornea. Preliminary experiments revealed no deposit in the corneal tissues when incubated in solutions with nucleic acid as substrate such

The nuclease preparations were generously supplied to us by Dr. Jessie Greenstein of the National Cancer Institute, Bethesda, Md.

as in previous experiments yielded satisfactory preparations with rat's kidney sections. It had been our habit in these experiments to incubate some 10-20 sections of tissue in 20 cc of substrate solution. Since the rat's cornea is less than 0.2 mm thick, the total volume of tissue in a cross section of cornea is very much less than in a cross section of kidney. It appeared possible, therefore, that some necessary co-factor for the enzymatic reactions might be eluted from the corneal sections. Experiments were therefore made with increasing numbers of corneal sections per cc of substrate solution, and it was found that as the relative volume of tissue to substrate solution increased, some activity began to appear in the preparations.

As a first step toward identification of such potential co-factors, a solution was prepared containing those elements of Tyrode solution which were not incompatible with the phosphatase reaction<sup>6</sup>. It was found that the addition of as little as 3 drops of this solution to 20 cc of substrate solution sufficed to activate the reaction (Fig. 3). Further experiments revealed that neither sodium chloride nor magnesium chloride were required in the activating solution. (It should be noted that the substrate solution itself contains abundant sodium and chloride ions.) Potassium chloride alone and glucose alone each yielded some activation but a more abundant deposit in the histochemical test was generally achieved when both these substances were added (Fig. 2). Maximal activation was achieved with glucose in a concentration of 0.01% in the substrate solution, and the activity was apparently unchanged by a further increase of glucose up to 0.1%. Maximal activation was achieved with KCl in a concentration of 0.003M in the substrate solution, and the activity was apparently unchanged by a further increase of KCl up to 0.3M. With reasonable assumptions regarding the concentration of glucose and potassium in the rat's kidney, one

<sup>6</sup> NaCl	0.8 gm
KCl	0.02 gm
MgCl <sub>2</sub>	0.01 gm
Glucose	0.1 gm
Water	100 cc

Calcium chloride was not included since this was already present in the substrate solution. Phosphate and bicarbonate were omitted as incompatible with the phosphatase reaction and because the substrate solution was already buffered with barbiturate.

can readily find that with 10-20 sections of rat's kidney each approximately 20 mm<sup>2</sup> in area and 25 microns thick suspended in a total substrate volume of 20 cc the necessary activating concentrations for these two substances would be approached. The fact that no or only feeble activity was demonstrable in corneal sections without the addition of glucose and potassium is evidence that in this tissue also the

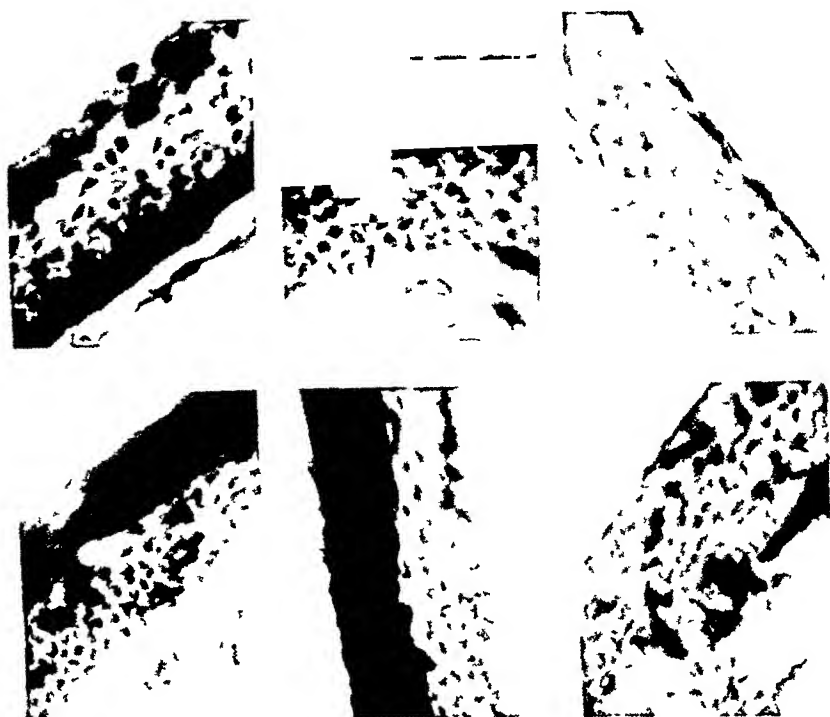


FIG. 1. Phosphatase activity in the rat's corneal epithelium with various substrates, each substrate used in concentration of 0.1%. Upper left, coenzyme I. Upper center, adenylic acid. Upper right, ATP. Lower left, hexosediphosphate. Lower center, sod. glycerophosphate. Lower right, ADP.

activity when demonstrable was not attributable to the non-specific alkaline phosphatase of Gomori. The latter enzyme does not require such activation and fresh frozen sections of rats' cornea show an abundant activity with glycerophosphate as substrate without the addition of glucose or potassium chloride to the substrate solution (Fig. 1).

The requirements for glucose and potassium supplement to the substrate solution given above were those which we regularly found when

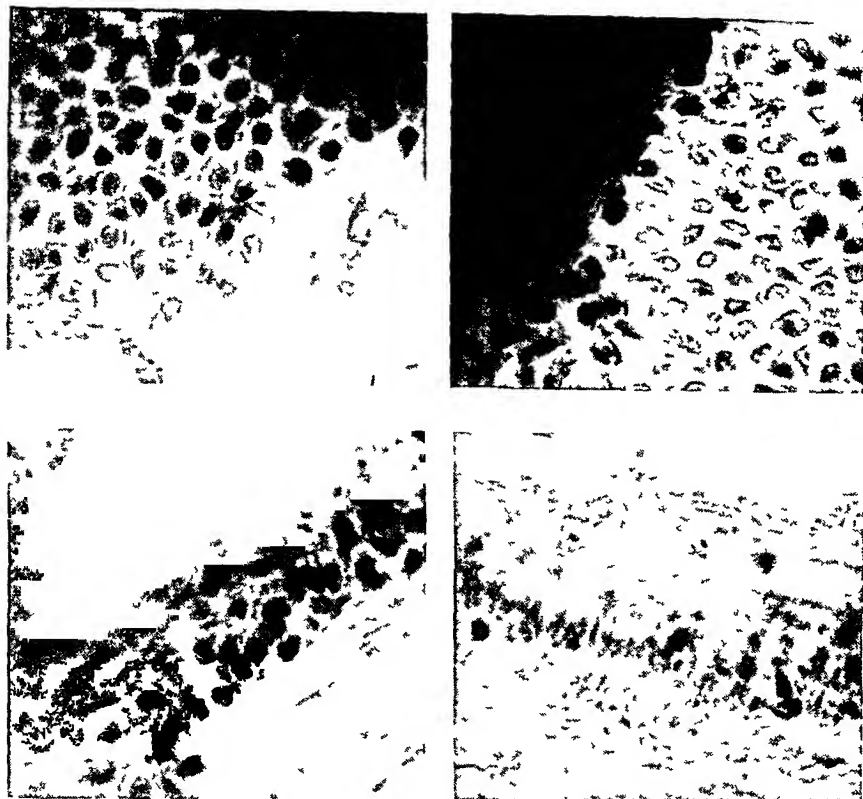


FIG 2 Ribonuclease activity in rat corneal epithelium pH 9.4 potassium and dextrose as activators Upper left, ribonucleic acid 0.1% + KCl 0.0001% Upper right, ribonucleic acid + dextrose 0.002% Lower left, ribonucleic acid + KCl 0.0001% + dextrose 0.001% Lower right, ribonucleic acid without KCl or dextrose

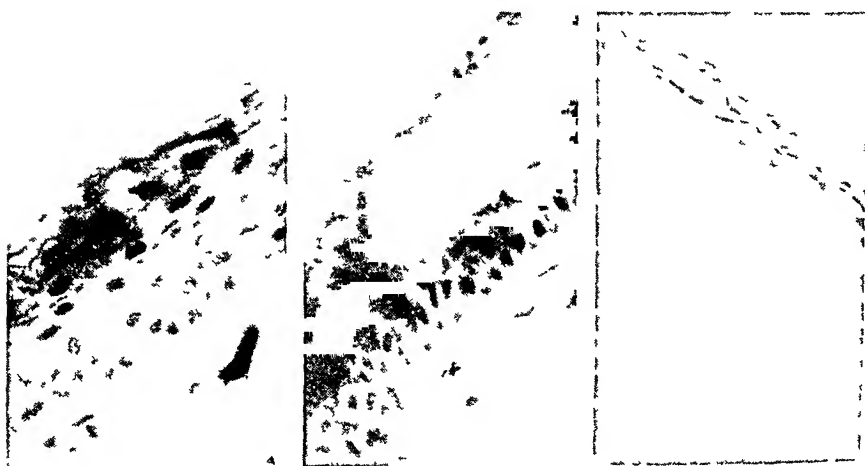


FIG 3 Phosphatase activity at pH 9.4 in rat corneal epithelium Tyrode as activator Left, thymonucleic acid + Tyrode Center, ribonucleic acid + Tyrode Right, Tyrode

animals were used that had been in our laboratory on a regular diet of Punna dog chow (Checkers), for a period of 2 weeks or more. When animals were used immediately on delivery from the supplier, the results in testing for nucleic acid phosphatase were often erratic in that activity could be demonstrated in some batches of animals without the addition of potassium and glucose to the substrate solution. It is to be remembered in this connection that the corneal epithelium contains glycogen. The erratic results on animals whose previous diet was uncontrolled may perhaps be connected with variation in the amount or availability of endogenous glycogen stores. We have made no attempt to analyze the possibility of such a nutritional factor being content, for the purposes of the present study, with the achievement of a reproducible method of demonstrating nucleic acid phosphatase activity. Under these conditions the deposit obtained on the histochemical test, and hence the presumptive site of enzymatic activity was, for ribose nucleic acid, both in the nucleus and in the cytoplasm of the corneal epithelium, whereas that for (depolymerized) desoxyribose nucleic acid activity, in the cornea as in the kidney, was limited to the nucleus.

The requirement of glucose as an activator for the splitting of phosphate from nucleic acid appeared to us most surprising. One would not expect that metabolic energy should be required for such a process. Moreover the concentration of glucose required for maximal activation was so very low (0.001%) that it could hardly be an effective source of energy if energy were required for the reaction. Greenstein, in studies on nucleic acid phosphatase in tissue brei, found that for some tissues the enzymatic activity was enhanced by preliminary dialysis and subsequent replacement of inorganic salts. The lower rate of liberation of inorganic phosphate in the undialysed brei indicates, as Greenstein (13) has pointed out, the presence in the brei of a diffusible substance which acts either as an inhibitor of the nucleic acid phosphatase or as an acceptor of the liberated phosphate. Accordingly Greenstein has suggested<sup>7</sup> that the activation which we find on addition of glucose might be due to a role of this substance as a link in a chain of phosphate transfer, accepting phosphate from nucleic acid and yielding it up as inorganic phosphate in the presence of hexokinase. A close linkage

<sup>7</sup> Personal communication

of the required set of transphosphorylases in the relatively undisturbed cellular structure in frozen sections might lead to an increase in the rate of liberation of inorganic phosphate in organized tissue, while in the disorganization of tissue brei, the presence of a phosphate acceptor might decrease the rate of inorganic phosphate liberation.

In order to explore these interesting possibilities it would be necessary to apply rigorously quantitative analyses to the rate of phosphate liberation under varying conditions, using techniques similar to those applied by Greenstein. Histochemical techniques are highly unsuited for quantitative analysis and can reveal only very large changes in the rate of phosphate liberation. The following experiments cannot, therefore, be regarded as conclusive but are, nevertheless, of possible interest.

To test whether the role of glucose as an activator of the phosphatase reaction was that of an energy donor or of a phosphate acceptor, we have compared the effects of adding hexose diphosphate in place of glucose to our substrate solution. In concentration of 0.1% to 0.01% hexose diphosphate acts as substrate for phosphatase yielding an appreciable deposit in the sections. With concentrations of 0.001% or lower, no visible deposit was obtained up to 24 hours of incubation. Such concentrations, however, corresponded to those in which glucose achieved its maximum activation when added to substrate solution containing 0.1% nucleic acid. Consequently we added 0.01% hexose diphosphate to the standard substrate solution of nucleic acid. Some increase in the deposit was produced by the hexose diphosphate when these preparations were compared with those containing neither glucose nor hexose diphosphate. However, the maximum activation achieved with hexose diphosphate in these concentrations was considerably less than achieved by glucose in equimolar concentration. Since hexose diphosphate itself acts as substrate in higher concentration, there can be no doubt as to its ability to penetrate within the cells of the frozen section. As an energy donor hexose diphosphate would be expected to be more effective, as a phosphate acceptor less effective than glucose. The results are in accord with the supposition that the role of glucose in this reaction is that of a phosphate acceptor.

Adenosine triphosphate,<sup>8</sup> adenosine diphosphate,<sup>9</sup> and muscle ade-

<sup>8</sup> Prepared from rabbit muscle by the method of Needham (11)

<sup>9</sup> Prepared from ATP by the method of Colowick and Kalckar (14)

nylic acid (Schwartz) were each tried in concentrations of 0.001% at which level they do not themselves serve as effective substrates. No effect was obtained with any of these agents added to substrate solution containing 0.1% nucleic acid either in the presence or in the absence of glucose. These substances are, therefore, either not required for the transphosphorylation process or, alternatively, are already present in the tissue in sufficient amounts.

If glucose acts as a link in a transphosphorylating system hexokinase would probably be involved in the reaction. Colowick and Price (15) reported that hexokinase was activated by reduced cozymase, inactivated by oxidized cozymase. In a subsequent note, Colowick (16) reported that he was unable to repeat the original experiments. No explanation has been offered so far for this discrepancy. We used a preparation of cozymase obtained from Schwartz (30% cozymase, 70% unidentified constituents) and found that on addition of this material to the substrate solution at a concentration of 0.001%, the rate of liberation of phosphate was enhanced if the "cozymase" had previously been reduced by hydrosulfite, and was diminished if the "cozymase" had previously been oxidized by methylene blue according to the method of Green and Dewan (17). The addition of the oxidizing or reducing agent itself to the substrate solution in corresponding amounts produced no measurable effect on the nucleic acid phosphatase reaction.

In testing various oxidizing and reducing agents on the nucleic acid phosphatase anomalous results were obtained with quinone and with ascorbic acid. Each of these produced an apparent activation of the phosphatase, but each when added to the control solution in the absence of organic phosphate substrate yielded a deposit in the tissue. The anomalous behavior of quinone appears probably due to the binding of quinone to the tissue, and the subsequent binding of cobalt ions by the quinone. The anomalous behavior of ascorbic acid to which attention has already been called by Lassek (18) appears to be due to the oxidation of ascorbic acid and the subsequent splitting off of oxalate. In the presence of calcium in the substrate solution calcium oxalate would be deposited. Subsequent treatment with cobalt nitrate and then with ammonium sulfide would give a deposit of cobalt sulfide in place of the calcium oxalate. The reaction is of interest as a possible means for the study of ascorbic acid oxidase in tissue sections.



## SUMMARY AND DISCUSSION

The application of the histochemical alkaline phosphatase technique of Gomori to fresh frozen sections reveals activity for ribose nucleic acid as substrate both in the nucleus and cytoplasm. With (depolymerized) desoxyribose nucleic acid as substrate, activity was found only in the nuclei.

Fresh frozen sections of rat's cornea showed no activity with either of these substrates in the absence of glucose and potassium chloride, but strong activity in the presence of very low concentrations of these two substances. Since the non-specific phosphomonoesterase does not require these activators in order to attack glycerophosphate, it is concluded that under the conditions of these experiments, the nucleic acids are attacked solely by enzyme systems specific for these substrates.

Our findings in respect to the activating influence of very small amount of glucose are in accord with the suggestion of Greenstein that the attack of the tissue on nucleic acid may involve as its first step a transphosphorylation rather than a simple dephosphorylation. The histochemical technique does not readily lend itself to such quantitative appraisal as is necessary in order to explore the mechanism of activation and further studies by other techniques are required. Nevertheless it should be pointed out that enzymatic histochemical tests applied to tissue in the relatively undisorganized state of fresh frozen sections can, potentially, reveal the integrated action of highly complex systems of enzymes that may be decomposed into apparently unrelated elements in disorganized tissue brei. Thus the histochemical technique, a blunt tool in respect to quantitative measurements can, nevertheless, furnish evidence of enzymatic integrations that are lost in the more conventional procedure.

The possibility of enzymatic transphosphorylation from nucleic acid to glucose raises interesting questions concerning the phosphate bond energy in nucleic acid, since a high energy bond would be required in order to phosphorylate glucose. The structure of nucleic acid proposed many years ago by Levene does not readily lend itself to the suggestion that some of the phosphate bonds may be of the high energy group. Recent studies by Gulland, however, indicate that the structure of nucleic acid is far more complex than that suggested by Levene. It is not necessary to assume, however, that energy required in a com-

plex transphosphorylation process must all be supplied by the phosphate bond initially split. In fresh frozen sections metabolic processes involving endogenous substrates are presumably still active, and may supply the required energy.

Finally it should be pointed out that, if, as we have indicated the dephosphorylation of nucleic acid under the conditions of our experiments involves a complex series of transphosphorylations, then the histochemical localization must refer to the final, not the first link in the chain.

## II EFFECT OF MITOTIC INHIBITORS

In a series of reports from this laboratory, the rat's corneal epithelium has been shown to be an exceedingly favorable test object for the study of mitotic activity (19, 20, 21). A large number of agents have been found which delay or inhibit the onset of mitosis in this tissue. The present investigation was undertaken to explore one possible biochemical mechanism of mitosis inhibition. The studies of Caspersson (22) have shown that the prophase of mitosis is characterized by a very active synthesis of desoxyribose nucleic acid. It is apparent from Caspersson's work that a cell unable to accomplish the normal synthesis and breakdown of this nucleic acid would be unable to undergo a normal mitotic division. It follows that some agents which inhibit mitosis may conceivably produce this effect through inhibiting desoxyribose nucleic acid synthesis.

The suggestion that this is the mechanism of mitosis inhibition by ionizing radiation has in fact been advanced by Hevesy (23). Using radioactive phosphorus as tracer, Hevesy measured the turnover of nucleic acid phosphorus in rats before and after exposure of the animals to X-radiation. He found that following exposure, the phosphate turnover in ribose nucleic acid was normal, but in desoxyribose nucleic acid was markedly reduced. While these results are strongly suggestive of the conclusion which Hevesy sought to draw from them, they cannot be taken as conclusive since, if desoxyribose nucleic acid synthesis should occur normally only during mitosis, then any inhibitor of mitosis should reduce the turnover of desoxyribose nucleic acid phosphate irrespective of the precise point at which the inhibitor interrupts the mitotic cycle, so long as that point anteceded the normal phase

of desoxyribose nucleic acid synthesis. Moreover, Brues (24) has recently reported that in the peculiar "mitotic death" which is seen in some cells after very heavy doses of X-radiation, there is an increase in Foelgen positive material, presumably desoxyribose nucleic acid, in the dying cell. These cells, exposed to ionizing radiation in doses far above those required to delay normal mitoses still are able to synthesize Foelgen positive material.

Bodenstem (25), applying Hevesy's argument to the nitrogen mustards, found that amphibian larvae after exposure to these agents showed much smaller gains in desoxyribose nucleic acid than did normal controls. The interpretation of these data involves the same difficulties as those of Hevesy. Moreover, the nitrogen mustards appear to have an even greater propensity to produce "mitotic death" with increased Foelgen positive intranuclear material than has ionizing radiation.

In view of these contradictory implications, it seemed desirable to attack this problem by a definite assay of some enzyme potentially concerned in nucleic acid synthesis, and to pursue the study, if possible, by a histochemical technique which might reveal differences between cells normally capable of mitotic division and cells no longer capable of this activity. Since there are no doubt many different enzymes involved in nucleic acid synthesis, failure to find a particular one of these enzymes inhibited after exposure to a mitosis inhibiting agent would not disprove Hevesy's hypothesis. A positive experimental result would, however, be conclusive. This argument led us to attempt to develop the histochemical technique for the demonstration of phosphatase activity with ribose and desoxyribose nucleic acids as substrates, and to apply this technique to rat's corneas with and without previous exposure of the tissue to agents capable of inhibiting mitosis in this tissue.

The histological technique has been reported in the previous section of this paper. In a series of experiments the effect of various mitosis inhibitors on the nucleic acid phosphatase reaction was tested. Rat's eyes were exposed *in vivo* to heavy doses of X-radiation, ultraviolet, and the nitrogen mustard  $\text{CH}_3\text{N}(\text{C}_2\text{H}_4\text{Cl})_2\text{HCl}$ . Eyes were removed at various times after exposure and frozen sections prepared from them were compared in respect to nucleic acid phosphatase activity with

sections from the unexposed opposite eye of the same animal. No difference in activity was found between the treated and the control eye for either ribose or (depolymerized) desoxyribose nucleic acid in the presence or absence of glucose and KCl.

Tests were made in which cocaine, adrenalin, or arsenic were added to the substrate solutions. No effect was found on the nucleic acid phosphatases of concentrations of cocaine or adrenalin far in excess of that required to inhibit mitosis. High concentrations of arsenite

$\frac{M}{200}$  produced a partial inhibition of activity for both nucleic acids.

The concentration of arsenite required for this effect was, however, considerably greater than that required to produce inhibition of mitosis *in vivo*. It is to be concluded that none of these agents inhibit mitosis through inhibition of the nucleic acid phosphatase.

#### CONCLUSIONS

Though the nucleic acid phosphatase system appears to be a complex and integrated mechanism, our experiments reveal no evidence of inhibition or disorganization of this system by those agents that are notorious for their capacity to inhibit mitosis. It is concluded that the particular mitosis inhibitors which we have tested do not inhibit mitosis through inhibiting or disorganizing the nucleic acid phosphatase system.

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# MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

MONDAY, MARCH 14, 1949—8 15 P M

HURD MEMORIAL HALL, THE JOHNS HOPKINS HOSPITAL

*Dr Folts* The first paper on this evening's program is by Drs Walker, Marshall, and Johnson, and the title is

## ELECTROCORTICOGRAPHY

BY DRS A E WALKER, H C JOHNSON AND C MARSHALL

Electrocorticography is the process of recording cortical potentials directly from the exposed brain at the time of operation, using cortical electrodes and an electroencephalograph. This technique has a number of applications.

Epileptic foci which can be localized only to a certain region of the brain by the use of scalp electrodes can be sharply localized to a definite area of cortex with cortical electrodes. Epileptic foci which do not show spontaneous spiking may be localized by the intravenous injection, or local application to the brain, of metrazol, together with simultaneous recording of the cortical electrical activity. The effectiveness of cortical resection of a focus is also demonstrated by electrocorticography.

Cerebral neoplasms not infrequently lie subcortically and are not apparent at the time of exposure of the brain. A tracing of the exposed cortex may show slow waves arising from the brain overlying the tumor.

Electrocorticography is a valuable tool in the study of the physiology of the cerebral cortex. An example of this is the outlining of sensory areas of the cortex by the recording of evoked potentials. This technique has been used to date only for the study of the somato-sensory areas, but it should have a much wider range of application in the future.

*Dr Folts* Dr Walker and his collaborators' paper is open for discussion. If there is no discussion of Dr Walker's paper the next paper will be by Dr Elliot Newman and Drs Kattus, Sinclair-Smith, Genecin, Sisson, and Monge. The title is "Studies on the Function of the Kidney in Cardiac Failure."

## STUDIES ON THE FUNCTION OF THE KIDNEY IN CARDIAC FAILURE

BY DRS ELLIOT NEWMAN, KATTUS, SINCLAIR-SMITH,  
GENECIN, SISSON AND MONGE

The purpose of this paper is to present and discuss some examples of studies by which we have attempted to describe the functional alterations of the kidney in cardiac failure.

Our studies are made in an attempt to describe the mechanism of the salt retention by the kidney by correlating the changes in renal function with the clinical and metabolic condition of the patient and to attempt to determine some of the factors bearing upon salt retention by a study of the effect of stress and drugs upon the kidney in normal subjects and patients with cardiac failure

Our first study was of a 37 year old colored woman with marked congestive failure due to rheumatic mitral and aortic valvular deformities. She had the murmurs of mitral stenosis and insufficiency and of aortic insufficiency. There was no evidence of active rheumatism. When admitted to the hospital she was markedly orthopnoeic and dyspnoeic at bed rest with distended neck veins and peripheral edema.

The first 40 days during weight loss, a fall in venous pressure and symptomatic improvement were associated with a rise in renal plasma flow and fall in filtration fraction to normal. There was no significant change in the glomerular filtration rate. Thereafter the patient was at home and deteriorated again. She returned to the dispensary and was observed to have increased weight and venous pressure with markedly reduced renal plasma flow with the same glomerular filtration rate.

The glomerular filtration rate was quite constant and within normal limits for this patient throughout the entire period of observation. Her normal glomerular filtration rate according to the standards of Homer Smith would be 100 cc /min. The range of her determinations of glomerular filtration rate was 85 to 115 cc /min.

During the weight loss, metabolic studies were performed on this patient. No medication except a maintenance dose of digitalis was given.

Loss of sodium and chloride were parallel to the drop in weight and venous pressure. At first the intake of sodium chloride was low (2 gm of sodium chloride), later the intake was raised by an additional 5 gm of sodium chloride. The increased intake had very little effect on the general course of sodium chloride balance, the kidney rapidly excreting the extra salt. All these changes in sodium and chloride balance and total output took place without any significant variations in the glomerular filtration rate.

We conclude from the facts so far that the regulation of sodium chloride excretion by the kidney in congestive failure is not correlated with changes in glomerular filtration rate during recovery, and that a normal glomerular filtration rate can be maintained.

Our second case study was a 30 year old man with a history of rheumatic heart disease. He had dyspnoea on slight exertion with minimal cyanosis. He had minimal but definite edema of the feet and an elevated venous pressure to 160 mm. His heart was enlarged to the left and right and the rhythm was auricular fibrillation. Enlargement of the left auricle and calcification of the mitral valves were demonstrated by x-ray. He had been taking digitoxin, 0.1 mgm a day, for several months, and this was continued with no other medication.

Throughout the period of balance study no significant change in these renal func-

tions occurred. The initial weight loss was accompanied by a fall in venous pressure from 160 mm to normal, and there was marked negative balance of sodium and chloride. Following this there was retention of sodium and chloride for several days. The total amount retained during this period was nearly equal to that lost previously. However, no change in weight accompanied this retention. During this phase the intake was changed from 3 gm of sodium chloride to 7 gm. The extra salt was promptly excreted with no alteration in the balance pattern. Later the patient was allowed up and given some exercise during the day, which was associated with some further sodium chloride retention. No significant gain or loss of potassium or nitrogen occurred in this patient.

We conclude from this study that a glomerular filtration rate 50% of normal and a renal plasma flow diminished to  $\frac{1}{3}$  normal did not prevent loss of edema and superfluous body sodium nor was there any correlation between either glomerular filtration rate or renal plasma flow and sodium chloride balance. This patient had persistent renal ischemia throughout his observation and was able to excrete stored body sodium as well as extra sodium added to his intake. The renal blood flow did not increase with compensation as in the first patient.

We conclude, then, that the function of the kidney in cardiac failure may be affected in many ways and that the metabolic effects on the tissues other than the kidney of inadequate output of the failing heart, may be complicated and profound. The patient must be studied not only at rest but under the stresses of daily activity. Only then can we obtain an integrated picture of the many inter-related factors involved in the production and retrogression of the congestion and edema. We have seen that ischemia of the kidney occurs which is presumably a reflection of inadequate cardiac output. This may cause some retention of sodium and chloride by diminishing glomerular filtration rate. But the ultimate responsibility rests with the specific selective reabsorptive mechanisms of the renal tubule cells whose activity must be sensitive to other factors beside the reduction in the amount of fluid presented to them by the diminished circulation. The effects of posture, exercise and humoral substances and the renal nerves have as yet to be adequately determined, and the nature of the metabolic injury to other tissues is a relatively unexplored phase of the problem.

*Dr Folles* Dr Newman and his collaborators' paper is open for discussion.

*Dr Fletcher* I think we are all interested to see Dr Newman throw the spotlight on the function of the renal tubules in this very important problem of cardiac failure. I was particularly interested to hear him mention the possible relationship between tubular function and the renal nerves. Two years ago in St Louis my colleagues, Dr Kriss and Dr Goldman, and myself more or less by mistake became concerned with the influence of the renal nerves upon renal tubular function. We had been interested in the effect of removing the left adrenal upon the function of the left kidney and might have saved ourselves some trouble if we



had paid a little more attention to a study of Dr E K Marshall's in 1919, which was mentioned by Dr Newman. He demonstrated that the effect of removal of the left adrenal upon function of the left kidney could be shown only insofar as one injured the nerves to the left kidney, particularly the splanchnic nerve, inadvertently during the process of adrenalectomy. We can report to Dr Marshall that the situation hasn't changed in the dog's kidney in the last twenty years, and we found that we could explain our findings after adrenalectomy as chiefly due to the injury we caused to the nerves during our operation. Our experiments consisted in putting cannulas in both ureters of anesthetized dogs and determining the effect of unilateral adrenalectomy upon the excretion of chloride and water. During the course of the observations, we obtained some evidence which was not conclusive but suggested that cutting the left splanchnic nerve increased the excretion of water and chloride by the left kidney, by direct effect on the tubule consequent to cutting the nerve. For although there was increased filtration of water and chloride secondary to the cutting of the nerve, this increased filtration was not enough in certain instances to explain the increased excretion of water and chloride. It is possible that splanchnicectomy interfered with nervous impulses to the renal tubules of these anesthetized dogs and resulted in decreased reabsorption of water and chloride by the tubules and in an increased excretion. There were alternative explanations for these findings and we could not be sure about the mechanism.

Now these experiments were in anesthetized dogs and I think it extremely questionable whether their possible implications can be transferred to the situation in man. I agree with Dr Newman it will be of interest to find out about the function of these nerves in their relationship to the renal tubule of man.

*Dr Andrus* As if cardiac failure weren't complicated enough from the circulatory standpoint, it has now become a metabolic abnormality. If I recall the circulation of the kidney correctly, the efferent flow from the glomerulus represents a large part of the flow to the tubule. How far does the reduction of the circulation to the tubules, or does it in any part, account for the abnormalities which might be observed?

*Dr Follis* Is there any other discussion of Dr Newman's paper? Dr Newman

*Dr Newman* We tried to ascribe the control of sodium and chloride excretion upon one circulatory factor after another in the kidney and every time we have been upset by the next case we studied. In the first case studied, there seemed to be a correlation between the metabolic balance of sodium and the renal plasma flow. In the second case, there was none, and since that time we have seen other cases with no correlation between renal ischemia and the metabolic balance of sodium and chloride. For a time we thought that the high filtration fraction had something to do with salt retention because fluid filtered through the glomerulus

at a high pressure means that the plasma reaching the tubules would be more concentrated and might increase the degree of reabsorption of salt and water by the tubules. But, we have seen in the second case the filtration fraction did not change although major changes in the sodium chloride balance occurred.

*Dr. Follis* Is there any other discussion? If not, the meeting is adjourned.

## BOOK REVIEWS

These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Bulletin

*Acclimatization in the Andes* By CARLOS MONGE *Baltimore, The Johns Hopkins Press, 1948, 130 + XIX pp*

The author, who is Director of the Institute of Andean Biology and quondam Dean of the Medical Faculty of the University of San Marcos, Lima, Peru, is known internationally for his broad studies in the biology of man and animals living on the high plateaus of the Andes. This little book, crowded with generous quotations from the records of early Spanish explorers, conquistadores, priests and rules, is an engrossing description of the "bio-climatic determinism" which shaped the course of Andean native societies. Compelling evidence is furnished to demonstrate that much social and labor legislation of both the Incan empire and the Spanish viceroys was directed toward preserving the racial acclimatization of Andean natives, won over many generations. Dr. Monge regrets the incongruity of the fact that, "The Republic is still unaware of the problem." This book is recommended highly as an example of the fruitful results and promising future of studies which combine biological, geographical and sociological technics.

J L L, Jr

*Aviation Medicine in Its Preventive Aspects* By JOHN F. FULTON *Oxford University Press, 1948, VIII, 174 pp*

These essays comprise the Heath Clark Lectures delivered by the author in 1947 at the London School of Hygiene and Tropical Medicine. The five lectures are devoted to the important problems of altitude, decompression sickness, explosive decompression, acceleration and crash. In his familiar enthusiastic and felicitous style, Dr. Fulton presents phases of the historical background, the classical experimentation and the recent investigations in these fields. It must be emphasized that these attractive essays are by no means complete reviews of the topics listed. The reader who has had experience in this special field may note and perhaps regret that the form of these lectures has prevented inclusion of a number of important and fundamental studies executed during World War II. These omissions, necessitated perhaps by the ambitious scope of these lectures, do not detract from the clear and exciting story which Dr. Fulton tells so well.

J L L, Jr

*Cancer of the Esophagus and Gastric Cardia* Edited by GEORGE T. PACK, B S, M D *St. Louis, C V Mosby Co., 1949*

This monograph is a symposium on the diagnosis and treatment of esophageal and gastric cancer originally published in the June, 1948 issue of *Surgery*. Arti-

cles are included by such outstanding authorities, among others, as Garlock, Ochsner, and Sweet. As Pack points out, the progress made in the treatment of cancer of the esophagus is one of the notable achievements in surgery of the past decade. This book should be of considerable interest to students of surgery and surgeons who are working in this field.

E S S

*Doctors of Infamy* By ALEXANDER MITSCHERLICH 172 pp \$3 00 Published by Henry Schuman, Publisher, 20 East 70th St., New York 21, N Y

This does not make for nice, pleasant reading. The subject matter does not allow it. Yet, it should be read by all who are interested in experimental medicine and by all who hope some time in some small way to add to the knowledge of diseases of man.

This is a factual, documented account of the cruel and useless experiments performed by some German "physicians" under the Nazi regime. Some of them would have been classified as "reputable scientists."

A number of us heard of the indirect evidence during the war that various antirickettsial drugs and vaccines were being tested on cases of epidemic typhus caused by inoculating typhus rickettsia into man. Here, however, is the entry (p 44) from a Dr Ding's journal dated April 24, 1943:

"Therapy tests, acridine granulate (A-Gr-2) and rutenol (R-2). To conduct these therapy tests with acridine granulate and rutenol, thirty persons (fifteen each) and nine controls were intravenously injected, each with 2 cc of fresh blood from typhus patients. All the test persons contracted very virulent cases of typhus."

The rickettsia were maintained at full virulence in man by continued passage in man. Some three to five "carriers" were inoculated per month. Their mortality was probably about 95%.

The comparative efficacy of different vaccines (unfortunately these are called "serums" in the text) was tested. Louse vaccine Weigl yolk sac (Cox-Gildemeister-Haagen method) and several others were used with ten persons as controls. The whole group was then infected in the presence of Prof Gildemeister. The overall test of the efficacy of the vaccines was as follows: Of some 90 controls, 40 died, whereas of 383 protected by vaccine, 66 died. This is a significant reduction of mortality and might be quoted as useful information. However, such information was obtained by Allied forces without recourse to such direct, crude experimentation. Furthermore the relative value of the different vaccines is not established.

A series of experiments on sulfonamide, bone grafting, cellulitis, and mustard gas were carried out under the direction of the Professor of Orthopedic Surgery at the University of Berlin. These involved the direct inoculation of bacteria, tiny fragments of wood and glass into wounds. These experiments were not considered adequate by the SS because they did not sufficiently approximate the conditions at the front, and the next group of experiments were ordered with the

stipulation that "it would be necessary to inflict actual gunshot wounds on the patients"

On page 71, a case history begins, "November 11, 1942 At 1800 hours, under the designation 'purolin', the patient received an injection of 1 cc of pus in which numerous streptococci chains had been observed under the microscope The injection was administered on the inside of the left thigh close to the abductor canal Late at night the patient complained of severe headache and drawing pains in the left thigh

Many other types of "experiments" are described and documented Exposure to cold, drinking of sea water, low pressure

This book is a valuable record, which cannot be read without its making the reader think a great deal about the purposes of experimental medicine

F B B

*Experimental Immunochemistry* By ELVIN A KABAT and MANFRED M MAYER  
Charles C Thomas, Springfield, Ill, 1948, \$8.75 XV + 567 pp, 88 figures

The authors have undertaken the important task of describing the experimental methods employed in immunochemistry, with emphasis upon quantitative chemical methods Part I deals in detail with methodology, and Part II with the applications and uses of quantitative immunochemical procedures Subsidiary to these sections is the material occupying the second half of the book This contains more or less detailed descriptions of certain chemical and physical methods and special procedures often used by the immunochemist (Part III), and directives for the preparation of selected variety of substances (Part IV)

Although *Experimental Immunochemistry* is primarily for the laboratory, it is more than a manual of procedures The discussions and comments, and the selected references, provide some of the common ground for the overlapping interests of the immunologist and the chemist

As Dr Michael Heidelberger states in his introductory comment, a complete treatise on immunochemistry is yet to be written In the meantime, Kabat and Mayer's book may earn for itself a useful position alongside the works of Landsteiner, Marrack, and Boyd which have become established as indispensable aids to the student in this important field of knowledge

B C

*Nursing Care of Neurosurgical Patients* By ROLAND M KLEMM Springfield, Illinois, Charles C Thomas, 1949, xii + 117 pp, 62 figures

This book presents a concise account of a neurosurgeon's view of nursing care for his patients The text covers the clinical phenomena of neurological disease with which the nurse should be familiar The various conditions amenable to neurosurgical operative treatment are briefly discussed A section on operative technique, illustrating the instruments and operating room arrangements for various surgical procedures, is particularly valuable An appendix describing

special nursing techniques such as enemas, packs, etc., will be of interest to many student nurses

The technical information of the book, aided by clear illustrations, makes a valuable background for a better understanding of the problems involved in the care of the neurosurgical patient. But the student nurse or recent graduate unacquainted with neurosurgical terminology and practice may be confused by the text. For example, on the opening page the author presents an illustration of the layers of the scalp showing the "skin, superficial fascia, aponeurosis, subaponeurotic connective tissue, pericranium, subpericranium, skull and dura mater." But in the text he refers to "the galea" without indicating that the galea aponeurotica is synonymous with "aponeurosis."

In a textbook, absolute accuracy on well established points, is a necessity for otherwise the student integrates in his thinking misinformation which may never be eradicated. Yet on page 5 "Anterior to the cerebellum is the medulla and in direct continuity and below is the pons. Immediately below the pons is the spinal cord." Loose thinking probably accounts for a number of colloquialisms used in the book. "The aphasic structures lie in an indefinite delimited area." (p. 4) "The glossopharyngeal and vagus are functionally closely related. The former influences taste on the posterior third of the tongue, acting soft palate noted as well as gag reflex. Aphasia disappears and hoarseness may be vagus involvement. Special examination of vocal cords may be indicated. Vagus pulse rate, respiration" (p. 26). Misspellings or typographical errors are rather common.

Written from a surgeon's viewpoint it is perhaps understandable that details of nursing care are underemphasized, but in a book having a title such as this one, it might be expected that some discussion of nursing principles be given rather than the subject dismissed as it is on p. 60 "In thoracic sympathectomies, the same as any chest case." As a further elaboration of this theme, the book does not indicate that a patient is more than a neurosurgical case. Certainly today, the individualization of nursing care to meet the demands of the patient's personality is an important factor in both pre- and post-operative surgical therapy.

The book is well illustrated with both black and white and colored drawings, in this regard, being superior to most nursing textbooks. The general appearance of the book is pleasing and a tribute to the publisher.

The book should be a useful reference for the nurse working with neurosurgical patients either on the ward or in the operating room, but it cannot be recommended as a textbook for the student nurse.

A E W

*Obstetrical Analgesia and Anesthesia* By FRANK F. SNYDER, M.D., Associate Professor of Obstetrics, Associate Professor of Anatomy, Harvard School of Medicine. W. B. Saunders Company, 1949.

For the reader who wishes documented evidence of the hazards of parturition, "Obstetrical Analgesia and Anesthesia" by Frank Snyder provides far more,

for the physician who wishes to apply this information into practical technics of safe control of obstetric pain, this book provides far less. The author, from a wide experience in three medical schools over a period of many years, presents the summations of his experiences and experiments upon the animal fetus in a well written compendium on the physiology and pathology of the respiratory system during the transition from the intrauterine to the extrauterine existence. This section comprises more than half of the book. It records the stillbirth and neonatal death rates in rats, rabbits, guinea pigs, and man, as related to the incidence of intrauterine pneumonia, atelectasis, asphyxia, and pharmacologic intoxication.

Doctors Snyder and Rosenfeld of the Department of Pharmacology of the Johns Hopkins University performed an ingenious experiment for prolonging the intrauterine gestation period of the rabbit. This was accomplished by the injection into the pregnant rabbit near term with urine from a pregnant human. Such injection initiated ovulation and formation of secondary corpora lutea. Such hormonal influence delayed labor by a week or more. These maternal animals were then prepared by transections of their spinal cords. Abdominal laparotomy permitted the pregnant uterus with intact blood supply in tact to come into view in a warm saline bath. Fetal rabbit movement, respiratory patterns, and nerve stimulus reactions could for the first time be accurately recorded. Incision of the uterus permitted the baby rabbits to come out into the saline bath while still attached to their placental circulation. Under such conditions it was possible to determine the effects of all sedatives, narcotics, and general anesthetic drugs on fetal physiology. It was found that the fetal respiratory system was most delicately sensitive to all of these agents. Morphine, nitrous oxide, barbiturates, paraldehyde, chloroform, and ether, were most depressing.

While such experiments presented interesting information for the fetal rabbit, it could definitely be considered inconclusive as a theoretical pattern of human reaction, since the fetal rabbit at birth is very much less developed to correspond to approximately the sixth intrauterine month of the human fetus. It is well known that fetuses in early months of gestation are much more resistant to anoxia than are those nearer term.

The chapter on respiration before birth embodying Doctors Snyder's and Rosenfeld's original work is the classic chapter of the text. The chapter on intrauterine pneumonia, while interesting, is in no way related to the subject of obstetrical analgesia and anesthesia. The chapters on atelectasis and asphyxia are of greater importance.

The second half of the book on the treatment of pain during labor is a 170-page inaccurate compilation of the literature, which presents good evidence that the author is neither anesthesiologist nor an experienced obstetrician with adequate experience with the drugs he describes. No physician who reads these chapters could treat the pain of labor and delivery with security of accurate information. He would have been so disarmed with the dangers of obstetric amnesia and analgesia presented in the first half that he would be confused and alarmed at the admonitions of the second half. Entirely too much space was devoted to mor-

phine, with and without scopolamine combinations, and entirely too little space was devoted to the barbiturates and the local anesthetics. An astounding omission was no mention of the anatomical pathways carrying the pains of parturition. No attention was paid to the control of pain and fear mechanisms as postulated by Grantley Dick Read. No illustrations were used to simplify understanding of complicated techniques. Only three lines were devoted to saddle block spinal anesthesia—a technique that is certainly being used to deliver 8 to 10 per cent of hospital babies. The evidence presented on the use of demerol is inconclusive. It is regrettable that such splendid fundamental work on the physiology of the newborn and the intrauterine fetus should be camouflaged under the nondescriptive title, and further deprecated by an outdated second half of the book.

Nevertheless, this text should be read by every obstetrician and pediatrician. Information recorded within it is of great importance in helping to determine some of the analgesia-anesthesia factors in hospital infant mortality conferences. It is hoped that the publishers of this text will rename it, and that the author will remove from it the insubstantial portions which should not interfere with the wide acceptance of the contributions made herein.

R A H

*Preoperative and Postoperative Care of Surgical Patients* By HUGH C. ILGEN-FRITZ, with foreword by URBAN MAES. St Louis, C V Mosby Co, 1948

The reviewer can appreciate what an immense amount of study and hard work the author put into the production of this almost encyclopedic book. Surgeons, surgical interns, and those in residency training will find much useful information in the book, and it would be good practice to have a copy on the shelf in each ward office. The author presents excellent discussions of the physiologic bases for the usual therapeutic measures, and adequately presents the details of the techniques of these measures. The pathogenesis of surgical complications is outlined, and means of prevention as well as for cure are given. A most helpful feature is the inclusion in each discussion of reference to recent original articles in the literature pertinent to the subject. When controversial matters are discussed, such as the therapy of postoperative thrombosis of veins, or early ambulation, justice has been done to the views of both sides, although the reader may sense that the author's sympathy is on the side of conservatism.

The volume is well printed and contains numerous clear illustrations. The author considers in detail the postoperative care of patients who have had thoracic, abdominal, gynecologic, and vascular surgery, but does not discuss the postoperative care of neurosurgical, otolaryngological, urological, or orthopedic patients, the treatment of burns, however, is included. Perhaps the book would be of more value to interns if all the surgical specialties were included. All in all, the reviewer believes this compendium of modern knowledge to be a useful and worthwhile contribution.

E S S



*Psychiatry in General Practice* By MELVIN W THORNER, M D , D Sc W B Saunders Co , Philadelphia and London, 1948

In discussing the purpose of the book, the author stresses the need for psychiatric publications which are relatively free from highly technical psychiatric "lingo" but sufficiently informative to be of practical use to the physician in the care of patients with psychiatric disorders. In organizing the book, he has all but abandoned standard classification by the use of such adjectives as "confused," "suspicious," "anxious," "queer," etc attached to the noun "people." This procedure is designed to lead the reader to look upon mental illness as a quantitative rather than a qualitative variable. Yet, it is also likely to be misleading. For example, neurotic reactions are discussed under "anxious people" even though it is a well-known fact that many of these people are not at all anxious. To call psychopathic personalities "queer and twisted people" adds little to the understanding of their personality disorders while it might confuse those readers who know that "twist-reactions" are frequently referred to as "para"-disorders. The greatest asset of the book is the reporting of the case abstracts. Rarely is there to be found in psychiatric literature a more colorful description of the lives of people which, at times, becomes so strong as to appear almost unbelievable. The author's literary skill is undeniable and makes up for the excessive length and occasional irrelevance of the details reported on. Unfortunately, the book's weakness is to be found in the chapter on therapy. Even though examples of brief psychotherapeutic methods and their successes are offered, the reader learns little about when to apply them. More specialized techniques are mentioned as they concerned physical methods, but one is left with the impression that the author considers intensive psychotherapeutic measures too theoretical to be of interest for the general practitioner.

The author's effort in writing the book is unquestionably a step in the right direction. It could have been more successful had more space been devoted to the illustration of treatment of patients suffering from psychiatric disorders which a competent general practitioner may be called upon to carry out. Such a text will probably have to be written by the psychiatrically trained practitioner himself rather than the psychiatrist whose experience is necessarily limited by his specialization and restricted to the more complex problems of the field.

E A

*The Digestive Tract in Roentgenology* By JACOB BUCKSTEIN, M D J B Lippincott Co , Philadelphia, 1948 Pp 889

As a reference text for students and those practitioners not personally engaged in the roentgen diagnosis of gastrointestinal disease, this volume is a worthy successor to its eminent predecessor of several decades ago. Each of the anatomical divisions of the alimentary tract is subdivided into chapters dealing with the more significant lesions of that structure. Discussion of these topics is largely from the viewpoint of roentgenographic diagnosis. Those techniques deemed most valuable by the author are thoroughly described.

Throughout the work, detailed case reports with accompanying illustrations of excellent quality are employed liberally. Frequently however, the inclusion of such profuse clinical data is at the expense of vital considerations, etiology, pathology, and particularly differential diagnosis. A notable example in this regard is the failure to emphasize the frequent association of pernicious anemia and gastric carcinoma.

Of major importance to the gastroenterologist are the enigmas posed by antral spasm simulating carcinoma and colonic diverticulitis suggesting neoplasm. To the reader who utilizes roentgen methods, omission of such problems by the author is disappointing, in view of his rich experience. Those unfamiliar with these problems may quite erroneously assume that the radiological features of these disease processes are characteristic. Nevertheless, Dr. Buckstein's materials are a meritorious addition to the medical literature and deserve careful perusal.

M W D

*The Mechanism of Abdominal Pain* By V J KINSELLA Sydney Australasian Medical Publishing Company Limited, 1948

In this monograph the author, a well-known surgeon of Sydney, Australia, attempts to establish the neuro-anatomical basis for the interpretation of pain arising from physiologic or pathologic disturbances of the intra-abdominal viscera. He believes "that the mechanism for splanchnic pain does not differ essentially from that for somatic pain." His theories and conclusions are based, in part, on clinical studies of patients upon whom he has carried out surgical procedures under local anesthesia. In general, however, the author's approach is largely historical and philosophical.

The reviewer sympathizes with those who would render more accurate the diagnosis of intra-abdominal pathology by the interpretation of the clinical complaints and signs. To this difficult field contributions are most welcome.

E S S

*The Renal Origin of Hypertension* By HARRY GOLDBLATT, M D, C M Published by Charles C Thomas, 1948, 126 pages

As the investigator who devised the method and performed the experiments forming the basis for the modern concept of the kidney's rôle in hypertension, Dr. Goldblatt writes with preeminent authority. In this monograph he summarizes his own work and that of others, integrating the various pieces of evidence into a carefully drawn picture of experimental renal hypertension. Figures illustrating a number of the fundamental experiments first published in various journals between 1934 and 1944, are reproduced here. Several chapters are devoted to the constituents of the humoral mechanism, and in the final chapter a number of similarities and certain differences between human essential and experimental renal hypertension are pointed out. This small volume is a clear exposition of the essential theory of the renal origin of hypertension and serves as an excellent point for orientation toward the problem. It is not a detailed analysis of all the work

related to that subject, nor is an extensive bibliography included, but the sources from which more complete information may be obtained are indicated

C B T

*The Rh Blood Groups and Their Clinical Effects* By P L MOLLISON, A E MOURANT and R R RACE

This small volume provides an excellent summary of present knowledge of the Rh factors and their clinical significance. The authors, who have made important contributions in this field, are well qualified to discuss the subject. Detailed information concerning the classification of the Rh groups, their clinical significance and method of Rh testing is given in concise form. The material is presented with remarkable clarity and brevity, considering the complexity of the subject.

C L C

*Your Child or Mine* By MARY LOUISE HART BURTON In collaboration with SAGE HOLTER JENNINGS Published by Coward-McCann, Inc

This book describes the history of five children representing the five types of cerebral palsy namely, the spastic, the athetoid, the ataxic, and the rigidity and tremor. It illustrates the problems both physical and psychological which occur in these types and does differentiate the problems as they show up in these different types. This book is valuable as an informational source to parents and others who are working with these children.

It is non-technical and accurately written.

W M P

## BOOKS RECEIVED FOR REVIEW

- Atlas of Peripheral Nerve Injuries* By WILLIAM R LYONS, Ph D , and BARNES WOODHALL, M D Published by W B Saunders, Phila 339 pp \$16 00
- Aviation Medicine* By JOHN F FULTON 174 pp \$3 50 Published by Oxford University Press, 114 Fifth Ave , New York 11, N Y
- Cardiac Catheterization in Congenital Heart Disease* By ANDRE COUNRAND, JANET S BALDWIN and AARON HIMMELSTEIN Published by the Commonwealth Fund, 41 East 57th Street, New York 22, N Y 108 pp \$4 00
- Child Psychiatry* Enlarged Second Edition By LEO KANNER 752 pp \$8 50 Published by Charles C Thomas, Springfield, Illinois
- Clinical Case-Taking* By GEORGE R HERMANN, 4th edition 240 pp \$3 50 Published by C V Mosby Co , St Louis, Missouri
- Current Therapy 1949* By HOWARD F CONN and a large list of Consulting Editors Published by W B Saunders, Phila 672 pp \$10 00
- Doctors of Infamy* By ALEXANDER MITSCHERLICH 172 pp \$3 00 Published by Henry Schuman, Publisher, 20 East 70th Street, New York 21, N Y
- Handbook of Diseases of the Skin* By RICHARD L SUTTON and RICHARD L SUTTON, JR 749 pp 1057 illus \$12 50 Published by C V Mosby Co , St Louis, Missouri
- Hindu Medicine* By HENRY R ZIMMER 201 pp \$4 00 Published by The Johns Hopkins Press, Homewood, Baltimore 18, Md
- Introduction to Human Anatomy* By CARL C FRANCIS Published by C V Mosby Co , St Louis 3, Missouri 470 pp \$5 50
- Neurological and Neurosurgical Nursing* By GUTIERREZ-MAHONEY and CARINI Published by C V Mosby, St Louis, Missouri 516 pp \$5 75
- Nursing Care of Neurosurgical Patients* By ROLAND M KLEMM Published by Charles C Thomas, Publisher, 301 E Lawrence Ave , Springfield, Illinois 142 pp 62 figures \$3 00
- Operating Room Technique*, 2nd edition By EDYTHE LOUISE ALEXANDER, R N 765 pp \$10 00 Published by C V Mosby Co , St Louis, Missouri
- Pain Syndromes* By BERNARD JODOVICH and WILLIAM BATES pp 357 \$6 00 Published by F A Davis Co , Phila 3, Pa
- Pharmacologic Principles of Medical Practice* By JOHN C KRANTZ, JR and C JELLEFF CARR pp 980 \$10 00 Published by The Williams & Wilkins Co , Baltimore, Md
- Posttraumatic Epilepsy* By A EARL WALKER 86 pp \$2 75 Published by Charles C Thomas, Publisher, 301 East Lawrence Ave , Springfield, Illinois
- Practical Aspects of Thyroid Disease* By GEORGE CRILE, JR Published by W B Saunders Co , Phila 355 pp 101 figures, \$6 00
- Safeguarding Motherhood* By SOL T DE LEE 135 pp \$2 00 Published by J B Lippincott Co , Phila , Pa

- Textbook for Almoners* By DOROTHY MANCHEE Published by The Williams & Wilkins Co, Baltimore, Md 466 pp \$7 50
- The Rh Blood Groups and their Clinical Effects* By P L MOLLISON, A E MOURANT and R R RACE Published by His Majesty's Stationery Office, London, England pp 74, 1s 6d net
- The Story of Scabies* By DR REUBEN FRIEDMAN pp 468 \$7 50 Published by Froben Press, Inc 1776 Broadway, New York 19, N Y
- Veterans Administration Technical Bulletins, Series 10* Published by the Veterans Administration, Washington 25, D C
- Campbell's Operative Orthopedics* By J S SPEED and HUGH SMITH, vols I & II 1643 pp \$30 00 Published by C V Mosby Co, St Louis 3, Missouri
- Rheumatic Fever* By SABRA S SADLER, R N, B S 151 pp 204 illus \$3 50 Published by J B Lippincott Co, Phila, Pa
- Vitamin A Requirement of Human Adults An Experimental Study of Vitamin A Deprivation in Man* Compiled by E M HUME and H A KREBS Published by His Majesty's Stationery Office 1949, 145 pp 3s

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